BIOLOGICAL STUDIES ON WOODY LEGUMINOUS INVADERS WITH SPECIAL REFERENCE TO *ACACIA MEARNSII*, *A. MELANOXYLON* AND *PARASERIANTHES LOPHANTHA*.

BY

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Promotor: Dr. C. Boucher

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and has not previously, in its entirety or in part, been submitted at any University for a degree.

13/1/1997

Date

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SUMMARY

The seed- and seedling biology of *Acacia mearnsii* De Wild., *A. melanoxylon* R. Br. and *Paraserianthes lophantha* (Willd.) Benth., which are important invasive woody legumes in the Fynbos Biome of South Africa, were investigated to compare their competitive potential.

The three species all produce water impermeable seeds with the percentage of dormant, viable seeds >90%. Diaspore characteristics, as well as a cafeteria experiment involving ants, confirmed that *A. mearnsii* is ant-dispersed and *A. melanoxylon* bird-dispersed, but that *P. lophantha* is not ant-dispersed, as was previously believed. Chemical composition of the seeds and the anatomy of the seed testa appear to be related to mode of dispersal of the species.

Seed are stimulated to germinate faster by wet heat (hot water) treatments than by dry heat treatments. Dry heat, however, is more detrimental to seed than wet heat at higher temperatures. *A. mearnsii* is more resistant to heat treatments while *A. melanoxylon* is more sensitive. *A. melanoxylon* is more sensitive to acid treatments, but the thick seed testa of *P. lophantha* makes it almost completely resistant to acid treatments.

Germination of all species takes place over a wide range of temperatures and is independent of light. *P. lophantha* has the widest germination temperature range while germination of *A. mearnsii* is inhibited by temperatures >30°C and germination of *A. melanoxylon* is inhibited by temperatures <10°C. *P. lophantha* appears to be more sensitive to desiccation after the imbibition period. The germination of all the species seem to be equally affected by moisture stress.

P. lophantha seedlings can establish from the deepest sowing depths, followed by *A. mearnsii* and then by *A. melanoxylon*. These differences could be attributed to seed size and the energy content of the seeds. *P. lophantha* seedlings outcompete the seedlings from the other two species under all moisture and light conditions. *P. lophantha* seedlings appear to be most shade resistant, but also most sensitive to moisture stress.

A standing burn appeared to reduce the soil seed bank of *A. mearnsii*, but seedling establishment and coppicing of adult plants after the fire exacerbated the problem. The burning of standing populations of woody leguminous invaders therefore appears not to be a viable control option.

Application of leaves of the species to pot-grown wheat and maize as mulches had no allelopathic effect on the crop plants and further investigations under field conditions is deserved.

The species are ranked according to their competitive ability, and therefore, invasive ability inferred from their seed- and seedling characteristics that were investigated in this study. It is concluded that *A. melanoxylon* has the greatest invasive ability, followed by *A. mearnsii* and then by *P. lophantha*.

OPSOMMING

Die saad- en saailingbiologie van *Acacia mearnsii* De Wild., *A. melanoxylon* R. Br. en *Paraserianthes lophantha* (Willd.) Benth., wat belangrike houtagtige peulplant indringers in die Fynbos Bioom van Suid-Afrika is, is ondersoek om hul kompetisievermoë in die saailingstadium te vergelyk.

Al drie spesies produseer hardskalige saad waarvan meer as 90% kiemkragtig is. Saadeienskappe, sowel as 'n kafeteria eksperiment met miere, het bevestig dat *A. mearnsii* deur miere versprei word en *A. melanoxylon* deur voëls. *P. lophantha* word egter nie, soos voorheen geglo is, deur miere versprei nie. Die chemiese samestelling van die saad en die anatomie van die saadhuid blyk verband te hou met die spesies se verspreidingsmeganismes.

Nat hitte (warmwater) behandelings stimuleer saadontkieming beter as droë hitte. Droë hitte is egter meer nadelig vir die saad by hoër temperature as nat hitte. Hittebehandeling het die minste effek op *A. mearnsii* gehad terwyl *A. melanoxylon* die mees sensitiewe was. *A. melanoxylon* is meer sensitief teenoor suurbehandeling, maar die dik saadhuid van *P. lophantha* maak dit feitlik heeltemal bestand teen suurbehandelings.

Ontkieming van al die spesies vind plaas oor 'n wye temperatuurreeks en is onafhanklik van lig. *P. lophantha* het die wydste temperatuurreeks vir ontkieming, terwyl *A. mearnsii* ontkieming by temperature hoër as 30°C geïnhibeer word en temperature laer as 10°C die ontkieming van *A. melanoxylon* inhibeer. *P. lophantha* blyk meer sensitief te wees vir uitdroging na die imbibisie fase. Vogstremming in die ontkiemingsfase het die ontkieming van al drie spesies ewe veel beïnvloed.

P. lophantha vestig die beste van dieper plantdieptes af, gevolg deur *A. mearnsii* en laastens *A. melanoxylon*. Hierdie verskille kan toegeskryf word aan saadgrootte en die energie-inhoud van die saad. Saailinge van *P. lophantha* groei vinniger as diè van die ander twee spesies onder alle vog- en ligtoestande. *P. lophantha* saailinge blyk die meeste skadu-bestand te wees, maar is ook die mees vatbare vir vogstremming.

Dit blyk dat die brand van 'n staande infestasie van *A. mearnsii* die saadbank verklein, maar die probleem word vererger deur die uitbreiding van die bogrondse

populasie a.g.v. saailingvestiging en die hergroei van volwasse plante. Brand van staande populasies houtagtige peulplante blyk daarom nie effektief te wees as beheermaatreël nie.

Toediening van blare van die spesies as 'n deklaag aan koring en mielies in potte het aangedui dat die blare geen allelopatiese effek op die gewasse het nie en dat verdere ondersoeke in die veld gedoen behoort te word.

Spesies is gerangskik volgens hul kompeteringsvermoë, en dus hul indringingspotensiaal, soos afgelei van hul saad- en saailingeienskappe wat in die studie ondersoek is. Die gevolgtrekking word gemaak dat *A. melanoxylon* die grootste indringingspotensiaal het gevolg deur *A. mearnsii* en dan deur *P. lophantha*.

CONTENTS

Chapter 1

Introduction

1.1 Rationale

The Fynbos Biome, which comprises some 75 000 km^2 at the southern tip of the African Continent (Moll & Bossi 1984), is famous for the richness and diversity of its flora (Bond & Goldblatt 1984; Low & Rebelo 1996). Man has been present in the Fynbos Biome for at least 600 000 years (Deacon 1986) and the impacts on the biome of the burning, hunting and stock grazing of these peoples, although profound have probably been smaller than those arising from man's activities since European colonization (Macdonald & Richardson 1986). Since the European colonization, settled agriculture and the culture of a wide variety of alien plants has led to the complete transformation of at least 68% of lowland ecosystems and about 10% of montane ecosystems (Moll & Bossi 1984).

Vegetation surveys indicate that the Fynbos Biome is more severely invaded by alien organisms than other South African biomes (Macdonald 1984). Several hypotheses have been proposed to explain why the Fynbos Biome is especially susceptible to invasion by alien organisms (Macdonald 1984, Macdonald & Jarman 1984, Richardson & Cowling 1992). Invasive alien trees and shrubs, especially those of the genera *Acacia, Hakea* and *Pinus*, dominate thousands of hectares of natural and semi-natural vegetation, significantly modifying communities and threatening many indigenous taxa with extinction (Richardson *et al.* 1992).

The major invasive leguminous shrubs and trees in the Fynbos Biome are *Acacia cyclops* and *A. saligna* which thrive in the lowland ecosystems, *A. longifolia* which mainly invades montane and riverine ecosystems, *A. melanoxylon* which invades indigenous forests, *A. mearnsii* and *Paraserianthes lophantha*, which both invade indigenous forests and riverine ecosystems (Macdonald & Jarman 1984).

This study was initiated to investigate aspects of the autecology of *A. mearnsii* De Wild. The study was initiated mainly as a result of the deadlock that existed between the Plant Protection Research Institute (PPRI) of the Department of Agriculture on the one hand, and the South African Wattle Growers Union (SAWGU) on the other hand,

regarding the introduction of biological control agents against *A. mearnsii*. Stubbings (1977) presented various arguments against the introduction of biocontrol agents and advocated the use of alternative chemical and manual control methods against *A. mearnsii*. These arguments probably contributed largely towards the refusal of the SAWGU to allow research on the biocontrol of *A. mearnsii* to be undertaken, as well as the decision of the PPRI to initiate the autecological study of *A. mearnsii*.

Later on, *A. melanoxylon* R. Br. and *P. lophantha* (Willd.) Benth, whose habitat overlap to a certain extent with those of *A. mearnsii* (Macdonald & Jarman 1984), were included in the study to compare certain aspects of the seed- and seedling ecology of the species. Co-occurring invasive species will probably compete with each other until, in the long run, one species will dominate the community. This process might happen slowly, over an extended period of time, or might be accelerated by natural factors such as fire, or by human interference. Human interference may take the form of a control programme against a selected species, which could allow another, more resistant species to dominate the community, as was the case with *A. longifolia* and *Hakea sericea* (Pieterse 1986). It is therefore of some importance to determine the relative competitive ability of co-occurring species. In the case of invasive species, the concept of competitive ability should also include the ability to resist control methods, as these may be a force influencing the outcome of competition.

Knowledge about the competitive ability of co-occurring invasive species might be helpful in attempting to prioritize species for development of specific control programmes. Various strategies might be employed, but where several invasive species co-occur, it might be sensible to direct control programmes towards the species which are most difficult to control, to prevent that species from becoming dominant when more susceptible species are suppressed by the control measures.

Natural competitive ability will, however, also play a role in the selection process of the most dominant species. The better competitor amongst the invasives will probably also be the better competitor against indigenous species. Therefore, natural competitive ability, as well as resistance to control measures, should be considered when rating invasive species in terms of invasive ability.

The seed- and seedling phases of the life cycle of a plant are most sensitive to environmental hazards and the highest mortality figures often occur during these phases (Fenner 1985). The outcome of competition between two plants is usually is determined in the seedling stage (Harper 1977). The invasive ability of a species, as defined earlier, in the seedling phase is therefore most important. In this thesis, especially in Part 1, the seed- and seedling ecology of these three species are investigated to determine their competitive ability, and thus invasive ability. In Chapter 7 and 8 some aspects of the control and utilization of the species are investigated and in Chapter 9 the species are ranked in terms of invasive ability by making use of and extrapolating from the data obtained in the foregoing chapters.

1.2 Description and ecology of *A. mearnsii*, *A. melanoxylon* and *P. lophantha*.

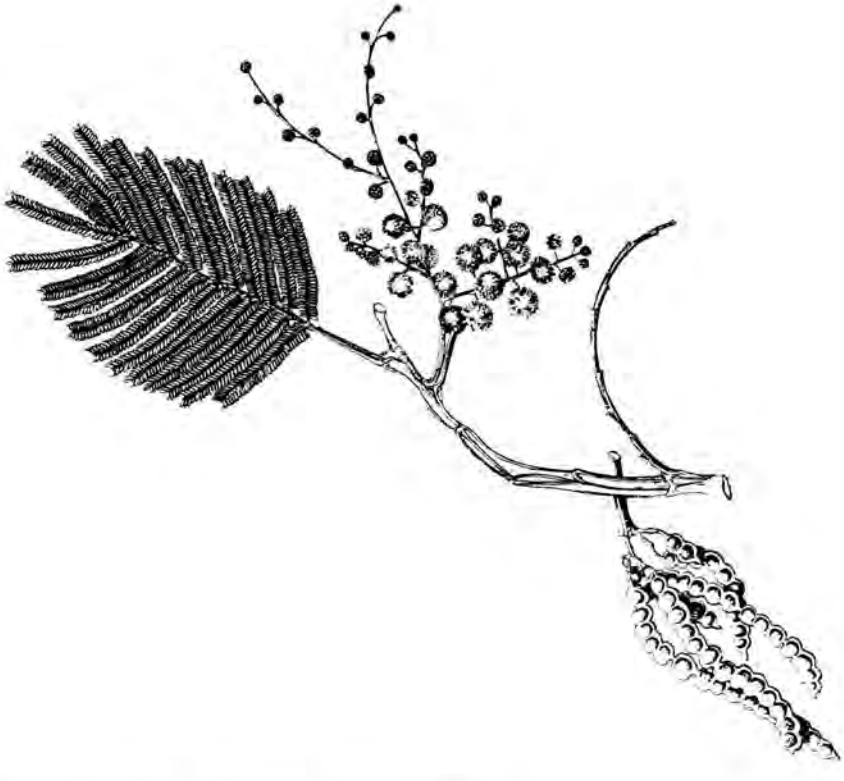

Figure 1. *Acacia mearnsii* (from Henderson *et al.* 1987)

1.2.1 Description

A. mearnsii is an unarmed tree up to 15 m high, with young branchlets having russet or golden pubescence. Leaves bipinnate up to 145 mm long, numerous raised glands

on upper surface of rachis, pinnae 8-21 pairs; leaflets 15-70 pairs up to 4 mm long and 0.75 mm broad, glabrous or pubescent below, margins usually ciliate. Flower-heads globose, in terminal panicles, pale yellow, strongly scented. Fruits flattish, jointed or slightly constricted between the seeds, straight or slightly curved, up to 100 mm long. Seeds black, 5 mm long and 3.5 mm broad, elliptic, compressed, smooth, caruncle conspicuous, seed stalk whitish yellow (Henderson *et al.* 1987). Leaves, flower heads and pods are shown in Figure 1.

Figure 2. *Acacia melanoxylon* (From Henderson *et al.* 1987)

A. melanoxylon is an unarmed tree up to 20 m high. Phyllodes finally glabrous, up to 120 mm long and 12 mm wide, linear-lanceolate to oblanceolate or narrowly obovate, straight to falcate, narrowed basally, with 3-7 prominent longitudinal nerves with characteristic net veining in between, particularly on young plants and coppice shoots. Hairy bipinnate leaves occur on phyllodes. Flower-heads globose, solitary or in short axillary racemes, pale yellowish white. Fruits oblong, falcate or variously

coiled or spirally twisted, up to 150 mm long and 8 mm wide, flattened, margins thickened, not constricted between the seeds. Seeds dark brownish black, compressed, smooth, 5 mm long and 2.5 mm broad, almost encircled by a dull red seed stalk (Henderson *et al.* 1987). Phyllodes, flower heads and pods are shown in Figure 2.

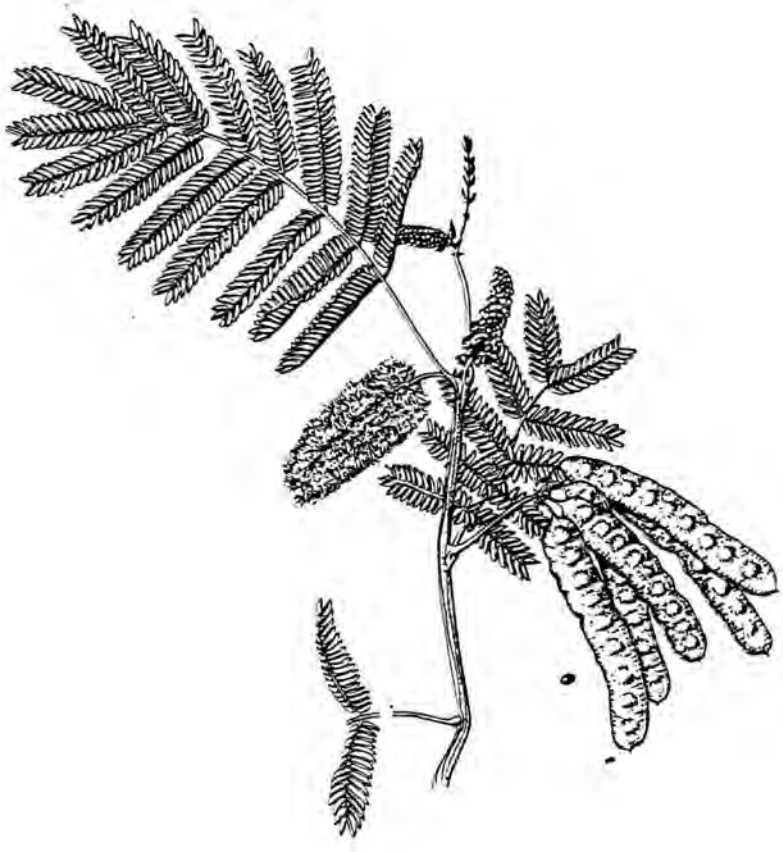

Figure 3. *Paraserianthes lophantha.* (From Henderson *et al.* 1987)

P. lophantha is an evergreen shrub or small tree usually 4-6 m high, but taller under favourable conditions. Branchlets fluted, minutely golden-haired when young. Leaves bipinnate, 140-750 mm long, a conspicuous elongated gland situated about midway on the petiole, pinnae in 7-12 pairs, leaflets in 20-35 pairs, 7-13 mm long and 1.75-3 mm wide, main vein markedly closer to upper margin. Flowers cream, in a dense head resembling a bottlebrush, 30-80 mm long and 30-50 mm wide. Fruit a flattened pod, 50-110 mm long, edges raised and thickened, end blunt but with a

distinct point (Henderson *et al.* 1987). Leaves, inflorescences and pods are shown in Figure 3.

1.2.2 Origin

A. mearnsii originates from south-eastern Australia occurring in areas of South Australia, Victoria, New South Wales and Tasmania. It may be found in areas where the annual rainfall is as low as 450 mm and as high as 1 500 mm but it appears to thrive best over the rainfall range between 625 and 875 mm. It occurs in areas that have an altitudinal range from sea level to about 870 m. At the highest levels it is subjected to severe frosts every winter, and not infrequently, to falls of snow, and moreover, it is often found in the open without the shelter afforded by an overstorey of eucalypts. It obviously possesses a considerable degree of cold-hardiness in its native habitat. In Australia *A. mearnsii* is found on a wide variety of soil types. It appears to thrive best on moist soils which are relatively deep, light, and well-drained, though it is often found on moderately heavy soils and occasionally on soils which are quite shallow. It tolerates a fairly high level of acidity but tends to avoid poorly drained or infertile soils. *A. mearnsii* is a pioneer species, but is present as a normal component of climax high forest (Sherry 1971).

A. melanoxylon originates from eastern Australia and Tasmania (Geldenhuys 1986). In eastern Australia it occurs at altitudes above 500 m at 17 °S but between sea level and 1 350 m in Tasmania at 43 °S (Farrell and Ashton 1978). It occurs in regions where rainfall means range from 450 to 1800 mm and areas where frosts are frequent and snow can be a regular feature. Deep humic soils, a high rainfall, and a plentiful supply of aerated ground water enhance vigorous growth. *A. melanoxylon* is a pioneer species in the rainforest succession in Australia. It is an important component of regrowth forest, but senesces and dies in the older regrowth stands and is absent from mature rainforest (Farrel & Ashton 1978).

P. lophantha originates from Western Australia. Not much is known about its ecology but it occurs in "Karri Forest" along roadsides, on seashore, sandy and lateritic soils (Nielsen *et al.* 1983b). This mesophytic understorey tree of the temperate forests has a frequency of distribution governed by the periodicity of fire (Dell 1980). It can therefore also be regarded as a pioneer species.

1.2.3 History, extent of invasion and weed status

It is generally believed that *A. mearnsii* was first established in South Africa in 1864 on the farm Camperdown in Natal (Sherry 1971) but evidence indicates that it was already present in Cape Town in 1858 (Boucher 1978). The Camperdown planting, however, was probably the main source of seeds that were spread into Natal and areas of Transvaal by transport riders and other travellers (Sherry 1971). Plantations were also established on the Cape Flats and at Kluitjieskraal near Wolseley in 1884 as well as in the eastern- and southern Cape around the turn of the century (Sherry 1971). Multiple importations of *A. mearnsii* seeds were made to Cape Town (Shaughnessy 1980). It is therefore possible that different races of *A. mearnsii* could have been introduced into South Africa. The information regarding *A. mearnsii* that was collected in this study could therefore only be applied to the *A. mearnsii* race that occurs in the southwestern Cape. *A. mearnsii* is rated as the eleventh, fifth and sixth most important invader in the Fynbos Biome (Macdonald & Jarman 1984), Natal (Macdonald & Jarman 1985) and Transvaal (Henderson & Musil 1984) respectively. Henderson (1992) ranked *A. mearnsii* the most important invader of streambank habitats in the Eastern Cape and second most important after *Opuntia ficus-indica* in roadside and veld habitats. *A. mearnsii* invades the Fynbos Biome, Grassland Biome and Savanna Biome in the Eastern Cape. Macdonald (1991b) found that *A. mearnsii* mostly invades riverine fringes in nature reserves in South Africa. The species is also a significant invader of grassland habitats and to a lesser extent, of forest edges. *A. mearnsii* was not only reported as invading more nature reserves (107) than any other species, but it was also ranked in the top ten species within four of the regions six biomes. On a national scale, *A. mearnsii* is probably the most important woody plant invader.

It is hard to obtain exact figures pertaining to the extent of the invasion of *A. mearnsii*. Van den Berg (1973) estimated that 47 900 ha country-wide was infested by *A. mearnsii* either as dense stands or as scattered individuals or along water-courses. In 1990 the estimated infestations of *A. mearnsii* and *A. dealbata* was more than six million hectares in the Cape Province and about nine million hectares in the

Transvaal (Coetsee 1990). The current distribution of *A. mearnsii* is depicted in Figure 4.

A. melanoxylon was introduced into South Africa from Australia in 1848 (Moll 1978). It was planted in the southern Cape forests from 1856 and later also in other parts of South Africa (Geldenhuys 1986). *A. melanoxylon* seed was again imported from a seed merchant in Paris (Shaughnessy 1980). Again, it is not certain whether all *A. melanoxylon* plants in South Africa belongs to the same race, and the data from this study is probably only applicable to the *A. melanoxylon* plants in the vicinity of Stellenbosch. *A. melanoxylon* is highly rated as a sawtimber, but also yields useful building poles, serviceable fence posts, and good firewood. In 1985 *A. melanoxylon* contributed 35% to the R0.75 million income from various timbers (Geldenhuys 1986). In the Fynbos Biome, *A. melanoxylon* was rated the tenth most important invasive vascular plant (Macdonald & Jarman 1984). In the eastern Cape, *A. melanoxylon* is locally common in the Amatole Mountains near Stutterheim and Hoggsback, but it does not present a big overall problem in that region (Henderson 1992). *A. melanoxylon* is present in Natal, but is not considered a serious invader by Henderson (1989). Macdonald and Jarman (1984), however, rated it thirteenth most important invader in Natal. *A. melanoxylon* is regarded as a less important species in the Transvaal, but it has a potentially wide distribution and small plants are often seen far from any plantings (Henderson & Musil 1984). *A. melanoxylon* has invaded 14% of 299 reserves and the highest percentage of biomes invaded is in the Fynbos, Grassland and Forest Biomes (Macdonald 1991b). In these reserves, *A. melanoxylon* is an important invader of forest habitats, and in particular, of forest edge and riverine fringe habitats (Macdonald 1991b). *A. melanoxylon* is a declared invasive plant (Government Gazette 1983). The current distribution of *A. melanoxylon* in South Africa is depicted in Figure 5.

P. lophantha was introduced to South Africa from Australia as a garden specimen by Baron von Ludwig in 1833. It was also sown on a farm near Claremont in 1834, and this was probably the locus from where the plant spread (Taylor 1978). It occurs mainly in the Fynbos Biome where it was ranked the ninth most important vascular invasive plant by Macdonald and Jarman (1984).

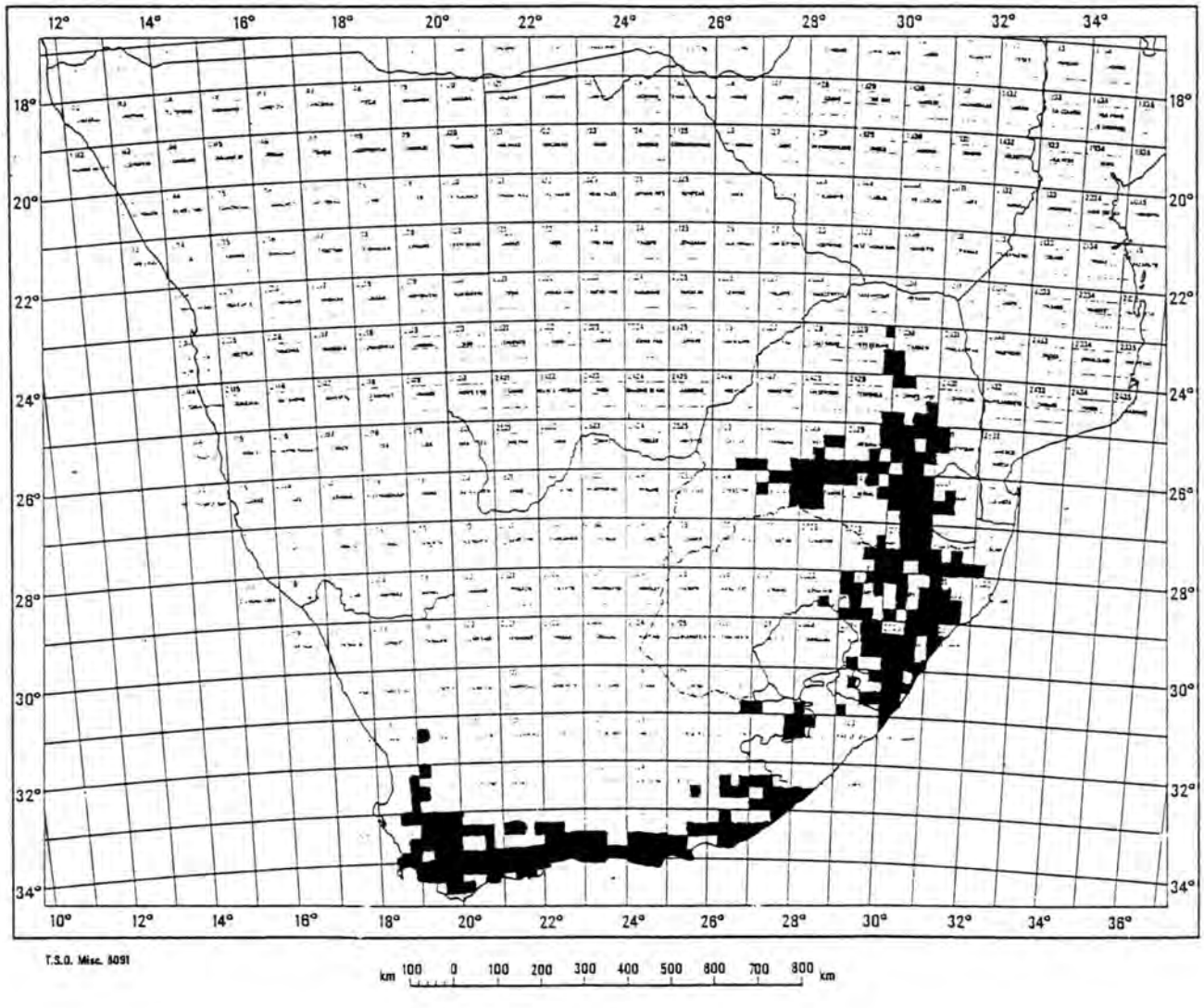

Figure 4. The distribution of *Acacia mearnsii* in South Africa. (Data from Henderson & Musil 1985; Henderson 1989; Henderson 1991; Henderson 1992; Henderson unpublished)

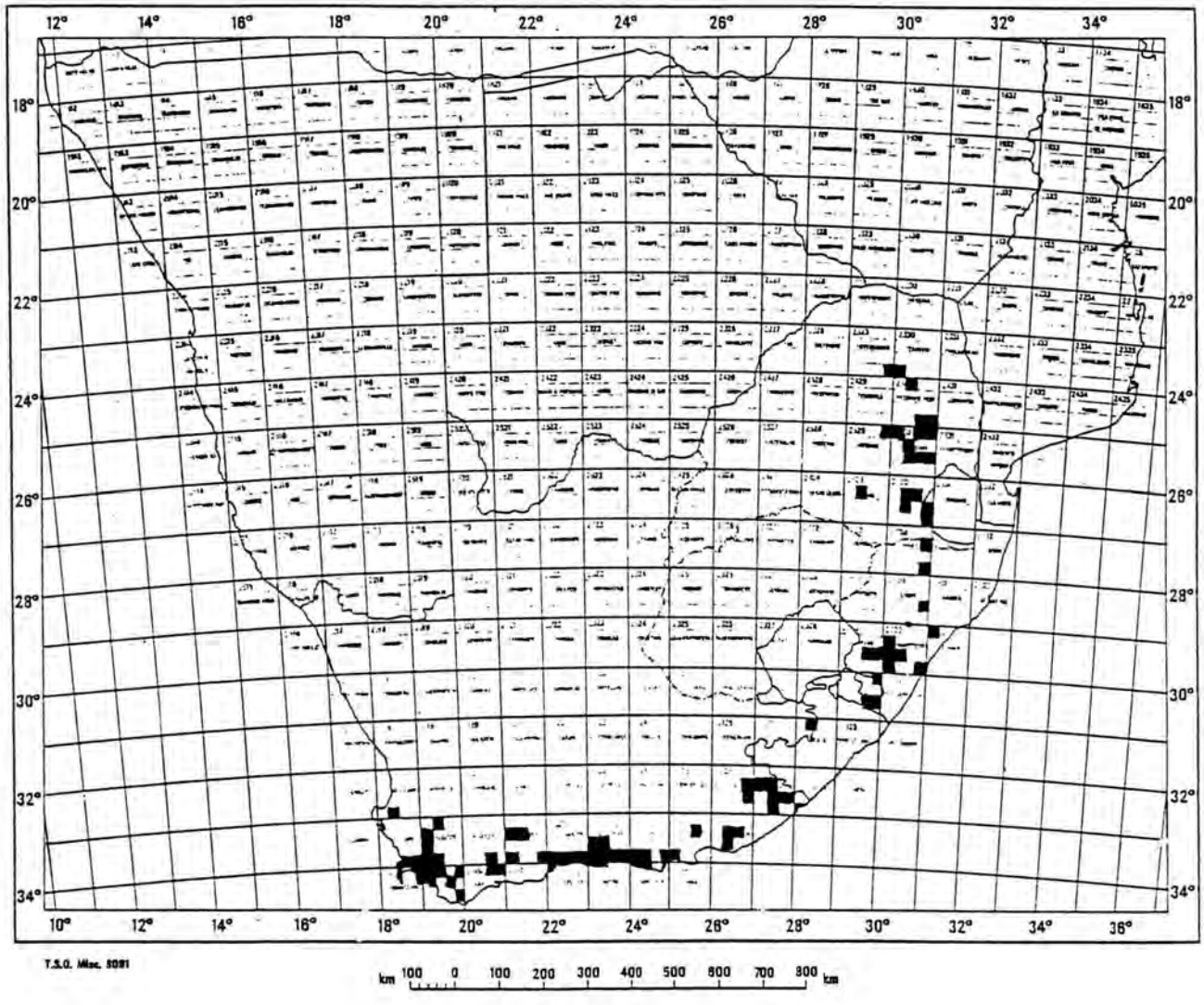

Figure 5. The distribution of *Acacia melanoxylon* in South Africa. (Data from Henderson & Musil 1985; Henderson 1989; Henderson 1991; Henderson 1992; Henderson unpublished)

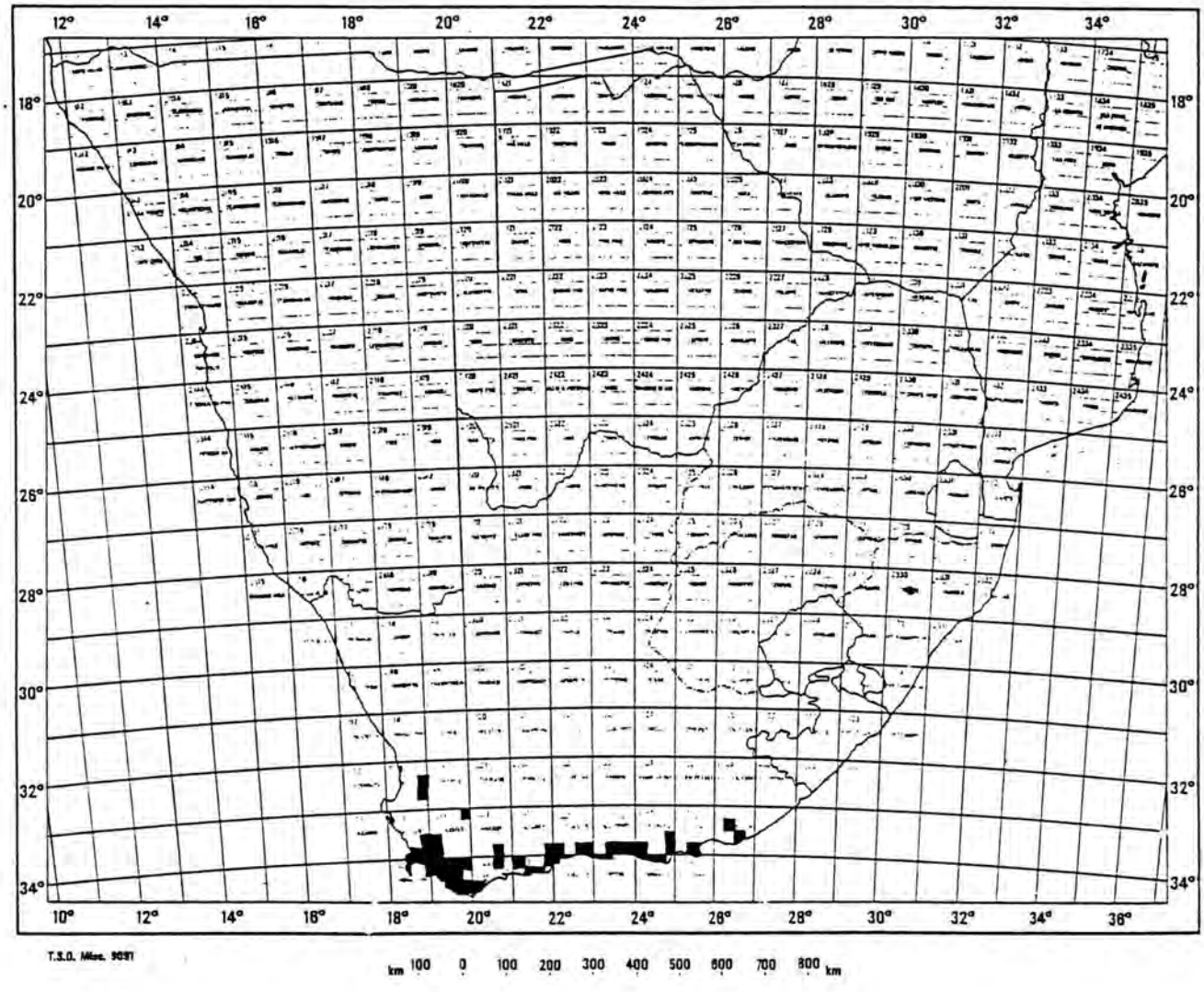

Figure 6. The distribution of *Paraserianthes lophantha* in South Africa. (Data from Henderson & Musil 1985; Henderson 1989; Henderson 1991; Henderson 1992; Henderson unpublished)

It is present but ranks low overall in the eastern Cape (Henderson 1992). It was also listed as present in Natal, but no ranking was given to it by Henderson (1989), so it can be assumed that it is relatively scarce in that region. It was not recorded in the Transvaal or Free State (Henderson & Musil 1984; Henderson 1990). It was only recorded in 4% of 299 reserves by Macdonald (1991b). Of these, it occurred in 12% of Fynbos, 3% in Savanna and 2% in Forest Biomes (Macdonald 1991b). It appears to be reasonably localized now, but it has the potential to invade more extensive areas (Taylor 1978). It is a declared noxious plant (Government Gazette 1983). The current distribution of *P. lophantha* in South Africa is depicted in Figure 6.

1.2.4 Negative impacts of the plants

The negative impacts of the three species will be discussed simultaneously because the species cause more or less the same problems. Jordaan and Van Zyl (1976) stressed the importance of *A. mearnsii* as an invader. They stated that *A. mearnsii, inter alia,* made ground useless for other vegetation, that it possibly exudes allelopathic substances, is responsible for a decrease in grass cover and a subsequent increase in erosion and is a luxury consumer of water. Most of these attributes may hold true for the other two species too.

To the authors knowledge, none of the species studied have been shown to have allelopathic properties, in contrast to *A. dealbata* (Casal *et al.* 1985) and *A. cyclops* (Jones *et al.* 1963) which have been shown to have allelopathic characteristics. The fact that *A. mearnsii* is recommended for crop rotations with maize or *E. curvula* (Anon. 1961, 1963) and that the sowing of *E. curvula* in the ashbeds of burnt *A. mearnsii* stands is recommended (Campbell 1987), shows that it cannot have a significant negative effect on other crops. No detrimental effects of *A. mearnsii* mulch on pot-grown maize and wheat plants were observed but *A. melanoxylon* could possibly have a negative effect on wheat growth (Chapter 8). Ver Elst (1996) found no negative effects of *A. mearnsii* mulch on field-grown wheat.

Most *Acacia* spp. enrich the soil on which they occur. Apart from nitrogen fixation by the mutualistic *Rhizobium* bacteria, *Acacia* spp. can also enrich the soil via excessive production of phosphorus- and nitrogen rich litter. Hallsworth (1958) estimated the total nitrogen input to an *A. mearnsii* plantation to be in the order of 20

g m^{-2} (200 kg ha^{-1}) p.a. *Acacia* spp., probably due to the presence of mycorrhiza, are also able to accumulate phosphorus. The enrichment of the soil by *Acacia* spp. can be a positive effect in cases where crops could follow the *A. mearnsii* stand on the same area, e.g. in grassland areas. However, in the Fynbos Biome, the enrichment of the soil could be detrimental to the growth of indigenous vegetation. The annual nitrogen and phosphate input by *Acacia* litter is about nine times that of fynbos communities and there can be little doubt that acacias make the soil increasingly unsuitable for Fynbos plants (Milton 1980b). It does, however, appear as if the pH of the substrate regulates the enrichment of soil by the *Acacia* species, soils with a low pH being not markedly enriched but calcareous soils with a higher pH being significantly enriched (Stock & Allsopp 1992). Musil (1993), surprisingly, came to the conclusion that the mineral enrichment of lowland fynbos soils by acacias has no detrimental effect on post-fire seedling growth and survival of indigenous taxa. Whether enrichment of fynbos soils is necessarily detrimental to the growth of fynbos species, is therefore a debatable point. In grassland, Beard and Darby (1951) found soils under *A. mearnsii* to have little more humus than grassland soils but the pH was 4.4 under *A. mearnsii* compared to consistently higher values around 5.3 for grassland.

As far as erosion is concerned, it was found that runoff under *A. mearnsii* plantations (7.7%) is higher than runoff in grassland (6%) (Beard 1956). Another, more noticeable negative effect of invasive *Acacia* spp., notably *A. mearnsii*, is the detrimental influence on river-bank erosion (Macdonald & Richardson 1986, Versfeld & Van Wilgen 1986). *A. mearnsii*, being shallow-rooted, is prone to collapse from bank erosion and in the face of floods. This exposes loose soil to further water action, while fallen stems divert the flow from the channel to the bank. Where the plants are felled, allowed to dry out and then burned, soil erosion is often accelerated on steep slopes (Cowling *et al.* 1976, Richardson & Van Wilgen 1986, Breytenbach 1989).

Bosch & Hewlett (1982), in a review of 94 catchment experiments world-wide, concluded that dense, tall vegetation causes a reduction in streamflow compared to less dense, lower vegetation. In South Africa a general reduction in streamflow was observed in several studies where indigenous scrub or grassland were replaced by *Pinus* spp. or *Eucalyptus* spp. (Nanni 1970, Bosch 1979, Van Lill *et al.* 1980, Van

Wyk 1987, Smith & Bosch 1989, Smith & Scott 1992, Chapman *et al.* 1995, Le Maitre *et al.* 1995). Beard (1963) observed that soils under grassland dried out progressively from the end of the rainy season until the rains of the next spring and were continuously saturated for 32 weeks in summer. Soils under *A. mearnsii* crops, however, dried out rapidly after rain and were desiccated far more in winter than soils under grass. In summer, soils were only saturated for two separate periods of 12 and eight weeks. However, indigenous forest had the same effects as *A. mearnsii* on soil moisture. Dye and Poulter (1995) found a 120% increase in streamflow shortly after clearing *Pinus* spp. and *A. mearnsii* from river beds near Lydenburg. There can thus be no doubt that *A. mearnsii* infestations will deplete soil water quicker and reduce streamflow compared to most indigenous vegetation types. The excessive water use of alien invasive plants relative to indigenous vegetation is the motivation for the initiation of the RDP-funded project where alien invasives in water catchment areas are being eradicated (Le Maitre *et al.* 1995).

Alien invasive plants also reduce bio-diversity of indigenous plants and are hazardous to endangered indigenous species, because they invade and alter the highly specialized habitat of these species (Stirton 1978).

1.2.5 Reproductive biology

In South Africa, *A. mearnsii* may begin to flower at about 20 months of age and ripe seed is occasionally found on three year-old trees. The period between fertilization and seed ripening is normally about 14 months (Sherry 1971). The seeds are water impermeable and are attached to an off-white funicle that contain elaiosomes, so that the plant is adapted to short distance dispersal by ants i.e. it is a myrmecochore (O'Dowd & Gill 1986). For long-range dispersal the plant is probably dependent on human transport (Macdonald 1991b) or it can be transported by water (Richardson & Cowling 1992). Being water impermeable, large soil-stored seed banks of up to 20 000 seeds can accumulate in the soil seed bank (Boucher 1978). The dormancy of the seeds can be broken by fires in the veld or by artificial heating procedures (Gupta & Thapliyal 1974; Hendry & Van Staden 1982). *A. mearnsii* plants usually coppice after burning or felling (Boucher 1978).

A. melanoxylon seedlings may become reproductive three years after establishment (Richardson & Cowling 1992). The seeds mature about three to four months after fertilization and are attached to a dull pink or red aril which is attractive to birds, resulting in the plant being classified as an ornithochore (Davidson & Morton 1984; O'Dowd & Gill 1986). In South Africa several bird species have been observed to spread the seeds of *A. melanoxylon* (Geldenhuys 1986; Geldenhuys *et al.* 1986) and water is also believed to be a major dispersal agent (Richardson & Cowling 1992). Seeds are also water-impermeable and can build up large seed banks in the soil (Geldenhuys 1986). Seeds can be stimulated to germinate by fire and artificial heat treatments (Gupta & Thapliyal 1974; Farrell & Ashton 1978). *A. melanoxylon* can coppice after burning or felling but can also form profuse amounts of root-suckers once the roots are exposed (Moll 1978).

P. lophantha can produce seeds within two years of germination (Taylor 1978). Seeds are also water-impermeable and can be stimulated to germinate by fire or artificial heat treatments (Dell 1980). The major dispersal agent is probably water (Richardson & Cowling 1992) although it has been classified as a myrmecochore by Pemberton and Irving (1990). *P. lophantha* does not coppice after burning or felling (Taylor 1978).

1.2.6 Control methods

Mechanical control

A. melanoxylon seedlings of up to 200 mm high can be handpulled, hoed or dug out. Plants with a stem diameter of up to 20 mm can be controlled by stripping the bark of the standing tree to the ground or by ringbarking as close as possible to the base of the stem or the base collar. The ring should be at least 100 mm wide in older plants and 10 mm in plants with a stem diameter of less than 20 mm. For plants with a stem diameter greater than 20 mm, bark stripping and ringbarking as described should be applied, as well as sawing off and applying chemicals to the trunk (Vermeulen 1989).

As mentioned earlier, *A. melanoxylon* coppices vigorously after felling or burning and it is imperative to apply herbicides to the cut stump. *A. mearnsii* and *P. lophantha* can be controlled by methods similar to those used for *A. melanoxylon*. Except for the largest trees, *A. mearnsii* stumps also coppice after felling or burning (Boucher 1978).

P. lophantha does not coppice and therefore cut stumps of this species need not be treated with chemicals. Campbell (1993) described various control strategies for use against *A. mearnsii* under different conditions. Some of those strategies can be used against *A. melanoxylon* and *P. lophantha*, conditions permitting.

Chemical control

No chemicals are registered for use against *A. melanoxylon* (Vermeulen *et al.* 1993) but herbicides registered for use against *A. saligna* and *A. cyclops* will probably be able to control *A. melanoxylon* as well. Experimental work on this topic is however necessary.

Triclopyr, alone or in combination with 2,4D or picloram, glyphosate, glyphosate trimesium and tebuthiuron are registered for use against *A. mearnsii* and details about their application are given by Vermeulen *et al.* (1993).

There are no herbicides registered against *P. lophantha* (Vermeulen *et al.* 1993). However, Pieterse (1994), recommended that glyphosate (180 kg l^{-1} a.i. - Sting®) at a concentration of 4% (v/v) be registered for spot spraying against *P. lophantha* plants of up to 2 m high. The same herbicide applied at 4 l ha^{-1} should also be efficient against smaller seedlings.

Biological control

A seed-feeding weevil, *Melanterius acaciae* was released against *A. melanoxylon* in 1986 (Dennill & Donnelly 1991) and causes considerable damage to the seeds (Donnelly 1995).

A seed-feeding weevil, *M. maculatus*, was released against *A. mearnsii* in 1994 but did not establish. A second release was made in August 1995 and it appears to have established at three sites (K. Stewart, pers. comm.). A fungus, *Cylindrobasidium laeve*, that kills resprouting *A. mearnsii* stumps has been identified and the possibility of developing a mycoherbicide from it is being investigated at present (Morris 1995).

Another seed-feeding weevil, *M. servulus*, was released against *P. lophantha* in 1989 and appears to have become established in the South Western Cape (Dennill & Donnelly 1991).

1.2.7 Conflict of interest

Conflict of interest regarding the control (more specifically, biological control) existed in the cases of *A. mearnsii* and *A. melanoxylon*, but not in the case of *P. lophantha*. The latter species does not appear to possess characteristics that would make it a desirable plant under any circumstances.

A. melanoxylon causes a threefold management conflict (Geldenhuys 1986). Firstly, it has become naturalized in open and frequently disturbed ecosystems outside forests which entails expensive control measures. Secondly, by meeting a strong demand it relieves pressure for the harvesting of indigenous timbers arising from the requirements of the furniture industry. Thirdly, it can be useful as a nurse tree for the rehabilitation of disturbed forest. A main factor why the conflict of interest could be resolved and permission granted for the release of a seed-feeding insect, is probably because the industry acknowledged the fact that *A. melanoxylon* is a pest under certain conditions, and as far back as 1982 an informal study group was formed to address the problem (Geldenhuys 1986).

In the case of *A. mearnsii*, Stubbings (1977) argued that it is not a pest plant as defined by Taylor (1969) *i.e.* an aggressive, introduced, woody perennial which forms a permanent cover and spreads unaided into natural plant communities. Stubbings insisted that *A. mearnsii* should rather be labelled a problem plant, which is a category of weed that has become temporarily dominant through poor application or complete neglect of the basic principle of veld and/or forest management. Coetsee (1990) quoted that the black wattle industry earns R65 million in foreign exchange and supply jobs for 22 000 people. It is therefore a valuable crop plant, but there is no doubt that *A. mearnsii* is a pest plant in areas outside those where it is cultivated, as was stated by Lückhoff in 1977. If it could have been proved at the outset that *A. mearnsii* was a pest plant in certain areas, the long and bitter conflict between PPRI and SAWGU could probably have been resolved far sooner.

1.3 STUDY FRAMEWORK

The thesis consists of 2 parts *viz.* Part 1 - A comparative study of seed, germination and seedling characteristics of three woody leguminous invaders, *A. mearnsii*, *A.*

melanoxylon and *P. lophantha* and Part 2 - Aspects of the control of woody leguminous invaders.

1.3.1 Part 1

Figure 7 depicts part of the life cycle of a typical vascular plant. Although not complete, the phases indicated in Figure 7 constitute some of the most important phases in the life cycle of a plant with regard to mortality factors. Pre- and post dispersal predation of seeds plays a major role in determining the fate of plant populations. The seeds that escape predation and end up in the soil, face more hazards. Each seed needs a special combination of conditions to be able to germinate. Some seeds possess the characteristic of dormancy, which enable them to survive adverse conditions. On the other hand, other seeds cannot withstand such conditions and may die, or germinate with the resulting death of the seedling under the existing adverse conditions. The germination requirements therefore can determine the survival or demise of a seed under natural conditions. Once the seed has reached a so-called safe site (Harper 1977), it will germinate and the seedling will then have to cope with predators, competition from other plants and environmental hazards such as drought, cold etc. Once the seedlings attain a certain age, which may differ between species and environmental conditions, the mortality risk will decrease. Therefore, the seed-germination-seedling phases are important in the life of a plant. The reaction of co-existing seeds to a certain set of environmental conditions will most likely determine the outcome of competition at community level.

In this study, these three phases will be investigated for the three species concerned. Knowledge obtained from these studies may shed light on the competitive abilities of the three plants concerned, the preferred habitat of the species, the adaptation of the species to certain conditions and the control possibilities for the three species concerned.

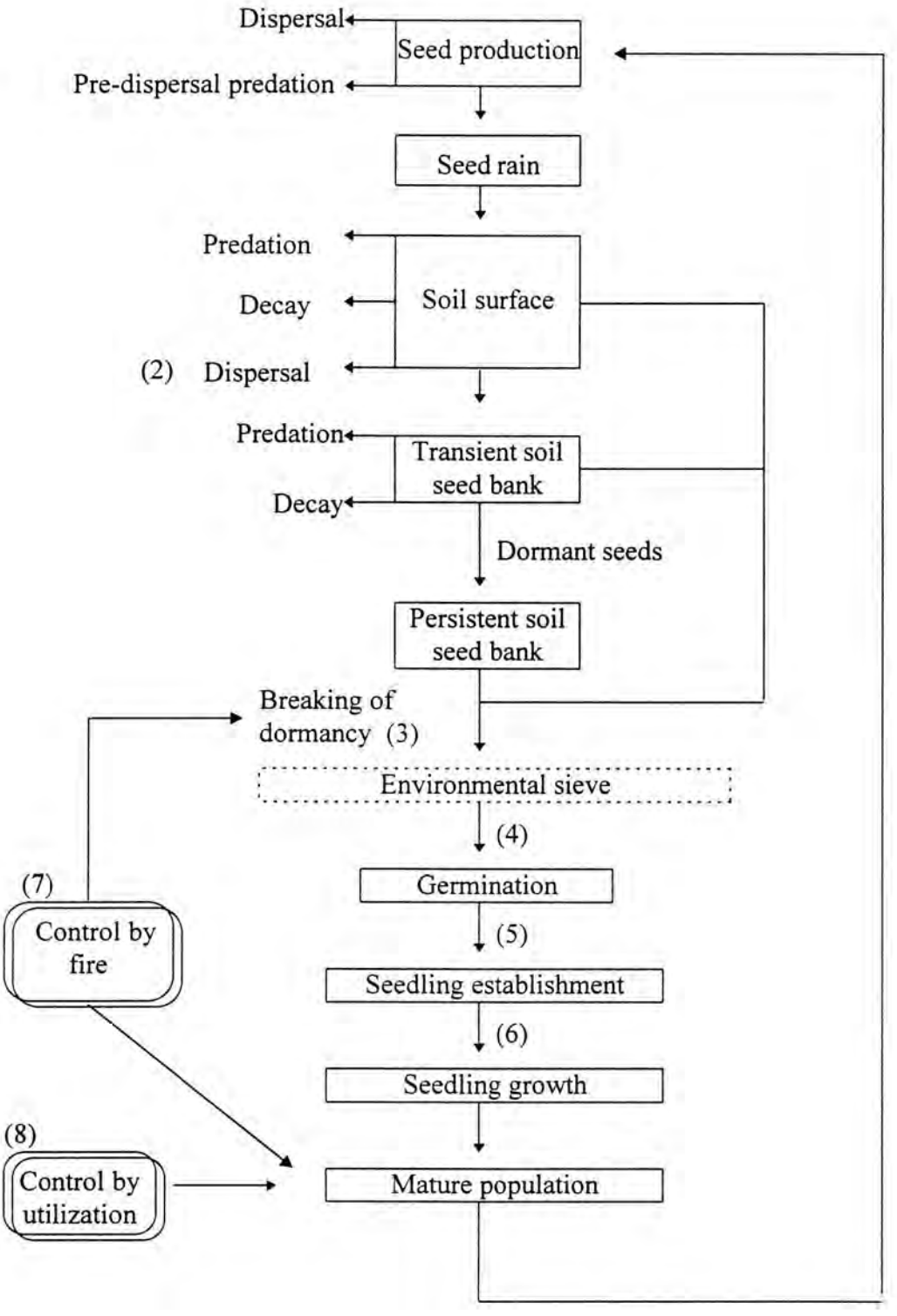

Figure 7. A simplified flow diagram of the life cycle of a hard-seeded leguminous plant. The numbers in brackets indicate the chapters that investigated specific aspects of the life cycle.

In Chapter 2 the seed characteristics of the three species with regard to morphology, dispersal adaptation and cell wall anatomy are investigated. Dispersal adaptations are important in helping to disperse seeds succesfully, because it has been shown that a high rate of spread is positively correlated to invasiveness of weeds (Forcella 1985). The cell wall anatomy of the seeds will probably influence the susceptibility of the seeds to dormancy-breaking mechanisms. Two hypotheses are tested *viz.*:

1) Seed dispersal characteristics of the species under investigation is similar in South Africa and in Australia;

2) Cell wall anatomy is not related to mode of dispersal.

Chapter 3 explores the germination requirements of the three species again trying to explain these with regard to the ecological niche of the species. Germination requirements may give an indication of which niches a species will prefer. A species which has a wide germination amplitude in terms of light, temperature and moisture will probably be able to invade a wide range of ecological habitats and will most probably be a more serious invader than species with more restricted germination requirements. The hypothesis tested is:

A. melanoxylon, originating from a less open forest habitat, will have more restricted germination requirements than the other two species, which occur in more open habitat in their country of origin.

In Chapter 4 the sensitivity of the seeds to different dormancy-breaking measures are investigated, and explained with regard to their ecological adaptations. The susceptibility of the seeds to dormancy-breaking mechanisms will influence the success of dispersal by *e.g.* birds, as well as their ability to resist control measures *e.g.* fire. The following hypothesis is tested:

Acid scarification will be a better dormancy-breaking mechanism for bird-dispersed species and heat will be better for ant-dispersed species.

The data collected in Chapter 4 may be useful when developing a control programme aimed at reducing the soil-stored seed bank of the species under investigation.

In Chapter 5 the transition from seeds to seedlings are investigated. Seed burial and seedling establishment from various germination depths will influence the growth of the seedlings after establishment. The ability of seedlings to establish succesfully from deeper germination depths might influence the outcome of competition between seedlings of these species, which form soil-stored seed banks. Two hypotheses are tested *viz.*

1) Large seeded species (*P. lophantha*) will be able to establish from deeper depths than the two smaller seeded species.

2) The two smaller seeded species will have a similar ability to establish from deeper germination depths due to their similar seed sizes.

In Chapter 6 the growth of the seedlings under varying stress conditions are monitored. The comparative growth of seedlings give an indication of the competitive ability of the species and the reaction of the seedlings to environmental stresses such as shading, drought and nutrient deficiencies also give an indication of the habitat where the species will survive. Three hypotheses are tested *viz.*

1) *P. lophantha*, having the largest seed and *A. melanoxylon*, originating from a forest environment, will be less influenced by shading than *A. mearnsii*;

2) *P. lophantha* and *A. mearnsii*, growing in a more open habitat, will be more drought resistant than *A. melanoxylon*;

3) *A. melanoxylon*, with a higher nutrient content in the seed, will be less influenced by low nutrient concentrations than the other two species.

Part 2

In Part 2 different aspects of control of the alien invader species are discussed. The controversial issue of burning as a control method against leguminous invaders is investigated in Chapter 7. Contrasting viewpoints about the use of fire in a control programme exist. It has been suggested that, where fire is used to reduce the soil-stored seed bank, the felling of the plants before the fire could be omitted in order to make the control programme economically more feasible. In Chapter 7 the following hypothesis is tested by investigating the effect of wildfire on an *A. mearnsii* infestation:

A standing burn can control an infestation of invaders succesfully.

In Chapter 8, a preliminary study of a method of control i.e. control by utilization is described. Utilization of twigs and leaves of invasive plants can also be considered as a control method, because it would reduce the biomass and leaf area, thereby reducing its water consumption, its ability to compete against indigenous plants and, if timed correctly, its reproductive output. In addition, if these trials prove to be successful, the invasives can then contribute towards the economical well-being of disadvantaged people by providing fuelwood and mulch. Mulching could reduce water loss, reduce levels of weed infestation and might, in the long run, add nitrogen to the soil and improve the physical properties of soil. Some *Acacia* spp., however, display allelopathic characteristics and in Chapter 8 the effect of mulch on pot grown wheat and maize is investigated. The hypothesis tested is:

> Addition of mulch will have no effect on the growth of pot-grown wheat and maize plants.

Lastly, in Chapter 9 the results from this study are discussed and used to rank the species according to invasive potential.

Part 1

A comparative study of the seed, germination and seedling characteristics of three woody invasive legumes

Chapter 2

Do seed-and seed testa characteristics reflect the dispersal adaptations of the species?

2.1 Introduction

The Fynbos Biome in South Africa which is famous for the richness and diversity of its plant species (Bond & Goldblatt 1984) is highly susceptible to invasion by alien trees and shrubs (Macdonald 1984). These plants can cause, amongst other problems, reduction of species richness of indigenous plant communities, extinction of rare Fynbos plants (Macdonald & Richardson 1986), and perhaps most importantly, a significant reduction in runoff from water catchment areas (Chapman *et al.* 1995). The effective control and management of these species is therefore of utmost importance.

The three important leguminous invaders of the Fynbos Biome discussed here are *Acacia mearnsii* De Wild., *A. melanoxylon* R. Br. and *Paraserianthes lophantha* (Willd.) Benth. (Macdonald & Jarman 1984). These three species prefer relatively similar habitats *i.e.* stream- and riverbanks and moist areas, although they can exist on drier mountain slopes (Macdonald & Jarman 1984). They are therefore important invaders and potential invaders of mountain catchment areas. The invasiveness of these and other leguminous species in South Africa is largely due to their prolific production of long-lived, hard-coated seeds (Milton 1980a). A sound knowledge of the seed- and seedling ecology of these plants is essential as the management of these large seed banks is a key element of an effective control programme (Holmes *et al.* 1987).

In this introductory chapter seed characteristics of the three species, that are naturalized in the south-western Cape, are investigated and, where possible, compared with information from their country of origin, Australia. This is done to ascertain whether any adaptations have taken place in South Africa in respect of seed characteristics. Two hypotheses regarding seed characteristics are tested *viz.* 1) Seed and testa characteristics are the same in South Africa and in Australia, and 2) there is no difference in seed testa characteristics of *A. melanoxylon*, a bird-dispersed species

in Australia (Davidson & Morton 1984; O' Dowd & Gill 1986), and *A. mearnsii* and *P. lophantha*, which are apparently ant-dispersed species (O' Dowd & Gill 1986; Pemberton & Irving 1990). In South Africa, water is regarded as the major dispersal agent for all three species (Richardson *et al.* 1992) although Geldenhuys (1986) indicated that birds play an important role in seed dispersal of *A. melanoxylon*.

2.2 Materials and methods

Fresh, ripe seeds were harvested directly from several trees in the vicinity of Stellenbosch (33°56'S; 18°52'E) during December 1987 and stored in plastic bins with lids until required. Seeds were again collected in December 1988 and used immediately to determine the dormancy and viability of the seeds.

Seed and aril characteristics

Seed and aril size

The length, width, thickness and mass of one hundred seeds of each species were determined and the means calculated. The wet mass of an additional one hundred seeds with arils, and the dry mass of the arils alone, were also determined. Because of the small size, 100 arils were weighed together, and the mass was divided by 100 to obtain the average mass of an aril.

Chemical composition

Entire seeds without arils, were milled and the chemical analyses indicated in Table 1 were performed. Standard methods for chemical analyses as prescribed by the AOAC, were used (AOAC, 1995).

Seed testa

Fifty seeds of each species were cut in half, perpendicular to the length axis, using a sharp blade and the thickness of the testa was determined at two locations around the perimeter of the seed by using a micrometer eyepiece on a stereo microscope.

Additionally, five seeds of each species were dipped into liquid nitrogen, cracked, coated with gold in an Edwards S150B sputter coater and the seed testas were investigated in a Topcon ABT-60 SEM.

For light microscopy, seeds were soaked in an ethylene diamine solution in the dark for 72 hours, dehydrated using the tertiary butanol method, embedded in wax and sections were made with a revolving microtome. Sections were stained using the safranin-fast green method (Sass 1958).

Dormancy and viability

Comparing two methods of determining viability

Twelve replicates of 25 seeds of each species were subjected to two treatments to compare two methods of testing for seed viability *viz.* the tetrazolium-chloride test for viability and germination tests. Seeds used in this experiment had been stored in sealed plastic bins for about 6-8 months. Some of the *P. lophantha* seeds possibly lost viability during storage because a portion of seeds were soft to the touch. They were nevertheless used because it suited the objective of the experiment. The viability of the *P. lophantha* seeds used in this experiment, is therefore not representative of the viability of freshly selected seeds of the species. The viability of the fresh *P. lophantha* seeds used in the next experiment, where seed dormancy of the three species was investigated, is therefore a better indication of the seed viability of the species. Seeds used in this experiment were nicked at the distal end to break dormancy before being subjected to the two treatments. In the tetrazolium-chloride treatment seeds were imbibed in distilled water for 24 hours at 20°C, then immersed into a 0.25% tetrazolium-chloride solution for three hours at 20°C before inspection (Moore 1973). For the germination treatment, seeds were put into a 90 mm diameter plastic petri-dish lined with 2 Whatman No. 1 filter papers. Six ml of a captab/benomyl mixture (0.055% captab and 0.022% benomyl m/v (a.i.)) (Clemens *et al.* 1977), was added to the petri-dish to prevent fungal contamination. The petri-dishes were enclosed in a polyethylene bag to conserve moisture and put into an incubator maintaining a constant temperature of 20°C. Petri-dishes were inspected daily until no further germination took place. Germinating seeds (a seed was recorded as having germinated when the radicle attained a length of at least 1 mm (Clemens *et al.* 1977)) were recorded and removed from the petri-dish. The mean number of viable seeds of each species for each treatment was calculated.

Dormancy

Six replicates of 25 seeds were incubated as above without being nicked. Germinated seeds were recorded and removed from the petri-dish every four days for 16 days. Thereafter all the seeds that were not imbibed, were nicked at the distal end and incubated for a further 8 days. From these data the viability and dormancy of the seeds were calculated. Data from the dormancy and viability experiments were logit-transformed before statistical analysis. Statistical analyses were performed by using the LSMEANS command of PROC GLM (SAS Institute Inc. 1985).

Attractiveness to ants

In order to determine the relative acceptability of the diaspores of the three species to ants, cafeteria experiments were performed in a disturbed Mountain Fynbos area outside Stellenbosch on three consecutive days in March 1996. Six ant nests were located within a radius of about 12 m in and around an abandoned gravel road. A pile of 10 diaspores of each species, were presented to the ants at each nest entrance. The piles were about 100 mm from the entrance and the piles of the different species were about 50 mm apart. The number of remaining diaspores were recorded every 30 minutes for a period of 120 minutes. After 120 minutes, the piles were replenished to 10 diaspores where necessary and were covered with a wire net (10 mm mesh) exclosure to protect the diaspores from other predators such as birds and rodents. Outside each exclosure another three piles of 10 diaspores of the three respective species were placed on the soil surface, not more than 200 mm from the protected diaspores. The diaspores were left overnight for a period of 20 hours before the number of remaining diaspores were recorded again. Fresh diaspores were presented to the ants in a similar manner as on the previous day and the removal of the diaspores were monitored every five minutes for 60 minutes, and thereafter every 15 minutes for the next 60 minutes. Again fresh diaspores were placed out under the exclosures for 20 hours, but this time no diaspores were left outside the exclosures. After 20 hours, on the following day, all remaining diaspores were recorded and collected, and any other diaspores or seeds (i.e. seeds with aril removed), except those remaining on the piles, were recorded.

To determine whether there were significant differences between the acceptability of the different diaspores, a separate ANOVA analysis of the number of diaspores remaining in each experiment was performed. Although strictly speaking, not correct, because different species of ants were involved, each ant nest was considered as a repetition in a completely randomized block design. Data were logit-transformed before analysis. At each nest, ants were trapped and immediately frozen until they could be identified.

2.3 Results

Seed and aril characteristics

Seed and aril size

A. melanoxylon has a dark, brownish black, smooth, compressed seed. *A. mearnsii* has a black, elliptic, compressed smooth seed and *P. lophantha* has a black, broadly elliptic, convex seed (Figure 1a,b). Although the shape differs, the seeds of *A. mearnsii* and *A. melanoxylon* are about the same size while the seeds of *P. lophantha* are considerably bigger (Table 1). The arils differ markedly - *A. melanoxylon* having a relatively large, red aril, *A. mearnsii* a smaller, off-white aril and *P. lophantha* a very small, brownish aril (Table 1, Figure 1a). The seeds of *A. melanoxylon* have a rectangular flattened appearance in cross-section while those of the other two species are more spherical in shape (Figure 1b).

Chemical composition

The most obvious features of the chemical composition of the seeds are that *A. melanoxylon* has a relatively high P, K, Ca, and crude protein content. *A. mearnsii* has a high oil content but a very low Mn content compared to the other two species (Table 1).

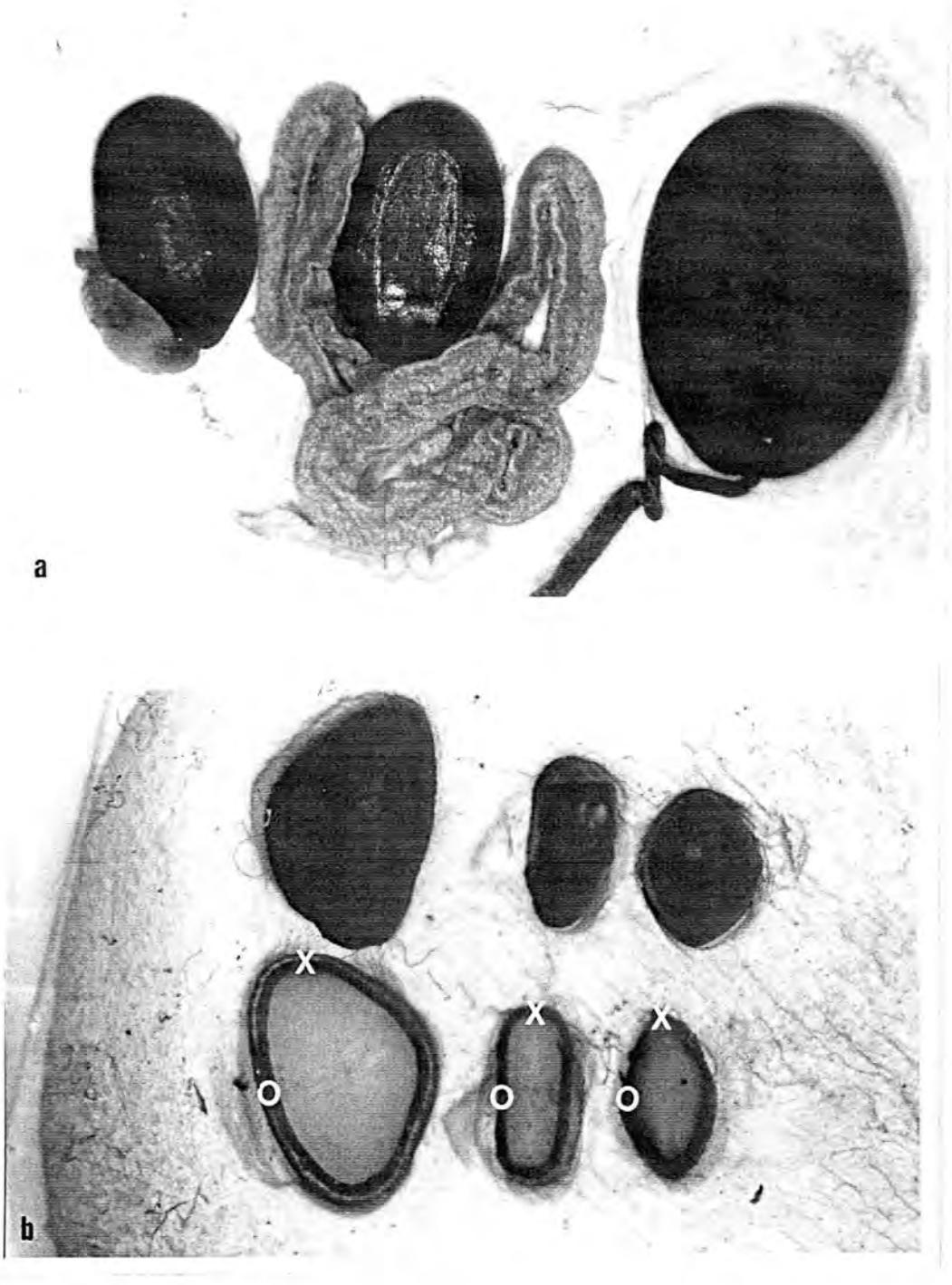

Figure 1 The diaspores of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. **a**. Whole seeds showing (from the left) the small off-white aril of *A. mearnsii*, the large red/pink aril of *A. melanoxylon* and the small brown aril of *P. lophantha*. b. Transverse sections of (from the left) *P. lophantha, A. melanoxylon* and *A. mearnsii*. The top row shows the distal end of the seeds. The bottom row shows the transverse sections of the seeds. **X** shows the spot where the measurements of the seed testa on the short side were taken and **O** shows the spot where the measurements on the long side were made.

Table 1 The size and chemical composition of seeds of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. (± indicate S.E.; * = calculated average of 100 arils weighed together; # = percentage investment in dispersal = aril dry mass/seed mass).

	A. mearnsii	*A. melanoxylon*	*P. lophantha*
Length (mm)	4.33±0.04	4.38±0.04	6.21±0.03
Width (mm)	3.00±0.02	2.99±0.02	4.71±0.03
Thickness (mm)	1.99±0.01	1.54±0.02	3.02±0.02
Seedmass (mg)	15.9±0.30	17.04±0.35	68.35±0.47
Dry aril mass (mg) *	0.86	4.63	0.35
Wet seed + aril mass (mg)	16.93±0.33	22.6±0.3	68.8±0.5
Investment (%)#	5.07	20.48	0.51
P (%)	0.14	0.34	0.22
K (%)	0.94	1.69	1.10
Ca (%)	0.20	0.27	0.19
Mg (%)	0.22	0.26	0.23
Na (mg/kg)	715	639	646
Cu (mg/kg)	5.92	12.8	7.7
Zn (mg/kg)	28.7	38.1	39.7
Mn (mg/kg)	72.2	229	227
Fe (mg/kg)	36	49.6	57.7
B (mg/kg)	23.1	22.9	23.4
Moisture (%)	7.5	8.26	7.07
Ash (%)	3.18	4.33	3.23
CP (%)	15.31	25.56	18.44
Oil (%)	10.12	5.24	5.29

Seed testa

A significant ($P<0.05$) interaction exists between the thickness of the seed testa of the three species and the side of the testa that is measured. In contrast with the other two species, the short sides of the *A. mearnsii* seed are the thinnest (Figure 2). The thinner area of the testa for the respective species are used as an indication of testa thickness. SEM micrographs show that the cuticle, palisade layer, hour glass layer and mesophyll layer are present in the seed testa of all three species (Figure 3a,b,c). The testa of both *A. mearnsii* and *P. lophantha* has a rough surface while the testa surface

of the *A. melanoxylon* seed is smooth (Figure 3d,e,f). *P. lophantha*, the biggest seed, has the thickest testa, as can be expected, while the seed testa of *A. melanoxylon* is thicker than that of *A. mearnsii* (Figure 2, Table 2). *A. melanoxylon* has a relatively thinner cuticle and palisade layer (34.3% of total testa thickness) than *A. mearnsii* (51.1% of total testa thickness) and *P. lophantha* (47.6% of total testa thickness), but a significantly (P=0.05) thicker mesophyll layer (61.4% of total testa thickness) (Table 2). *P. lophantha* has by far the thinnest cuticle (6.9% of total testa thickness) (Table 2).

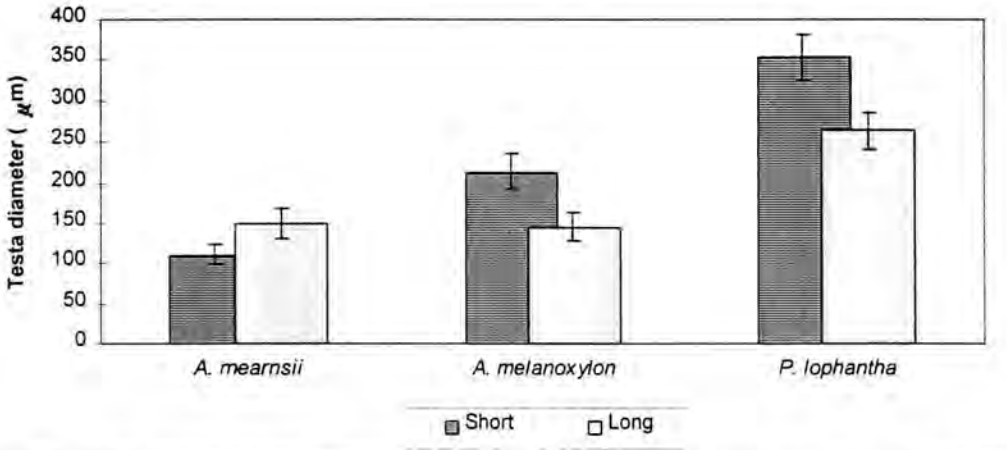

Figure 2 The thickness of the testa of seeds of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* measured at the short and long side of the transverse sections of the rectangular/elliptic seeds (see Figure 1 for explanation). (Vertical bars indicate standard deviation).

Light microscope sections show that *A. mearnsii* has a thick red-coloured cuticle on the outside and that the upper part of the palisade layer, which consists mainly of cellulose, is embedded into the cuticular material (Figure 3g). The hour glass cells are lignified while the mesophyl is mainly composed of cellulose. *A. melanoxylon* has the same composition, except for the mesophyl layer which is redder in colour indicating a higher lignin content than the mesophyl layer of *A. mearnsii* (Figure 3h). In *P. lophantha* the mesophyl layer is also lignified, but the hour glass cells are not lignified to the same extent (Figure 3i). The top of the hour glass cells however are embedded in a red material (*) into which the bottom part of the palisade cells are also embedded. The palisade cells of *P. lophantha* differ markedly from the other two species. The bottom parts of the cells conform to those of the other two species in

that they consist mainly of cellulose, although the top part, above the light line, is strongly lignified. Inner epidermal hour glass cells are conspicuous in *A. melanoxylon*, less conspicuous in *A. mearnsii* and appear to be absent in *P. lophantha* (Figure 3g-i).

Table 2 Dimensions of the different layers of the seed testa of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* (± indicate S.E.).

	A. mearnsii	*A. melanoxylon*	*P. lophantha*
Cuticle (μm)	31.5±2.4	24.5±1.5	27.9±2.1
Palisade (μm)	71.5±5.0	65.24±5.1	163.8±13.7
Hour glass (μm)	8.3±1.5	11.2±0.5	25.9±2.7
Mesophyl (μm)	91.7±9.7	161.1±8.7	182.9±10.9
Total (μm)	203.0±14.1	262.0±9.2	400.5±19.0
Cuticle (%)	15.5±0.7	9.4±0.8	6.9±0.3
Palisade (%)	35.6±2.5	24.9±1.7	40.7±2.3
Hour glass (%)	4.1±0.7	4.3±0.2	6.4±0.5
Mesophyl (%)	44.8±2.8	61.4±1.9	46.0±3.0

Viability and dormancy

Viability

There are no significant interactions between species and method of viability testing ($P=0.0527$) and no significant differences between the two viability testing treatments either ($P=0.1924$). There are, however, differences between species ($P<0.001$) (Figure 4) when 8 month old seeds were used. *P. lophantha* seeds are significantly less viable than the other two species, probably due to fungal attack during storage. In the next experiment, however, where dormancy and viability of fresh seed were determined, no significant differences ($P=0.056$) between species are observed (Figure 5).

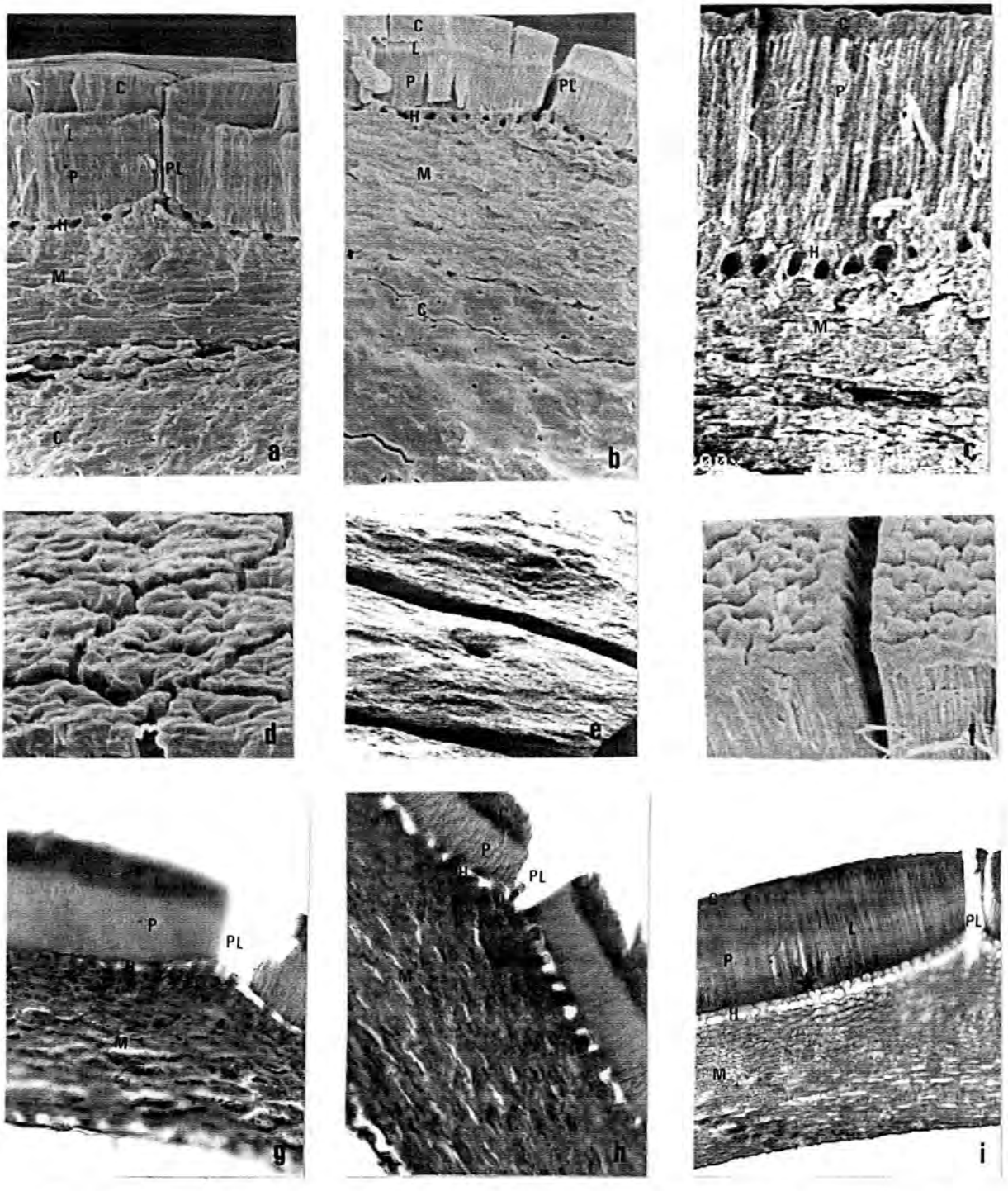

Figure 3 The seed testa of *Acacia meansii, A. melanoxylon* and *Paraserianthes lophantha.* **a.** Scanning electron micrograph of the seed testa of *A. mearnsii* x200. (C, cuticle; P, palisade layer; L, light line; H, hour glass cells; M, mesophyl,; C, cotyledon; PL, pleurogram). **b.** Scanning electron micrograph of the seed testa of *A. melanoxylon* x300. **c.** Scanning electron micrograph of the seed testa of *P. lophantha* x200. **d.** Scanning electron micrograph showing the deeply folded cuticle of *A. mearnsii* x800. **e.** Scanning electron micrograph showing the smooth cuticle surface of *A. melanoxylon* x1250. **f.** Scanning electron micrograph showing the folded cuticle of *P. lophantha* x200. **g.** Light microscope micrograph of the seed testa of *A. mearnsii* x200 (Note the inner layer of hour glass cells I). **h.** Light microscope micrograph of the seed testa of *A. melanoxylon* x200. **i.** Light microscope micrograph of the seed testa of *P. lophantha* x100.

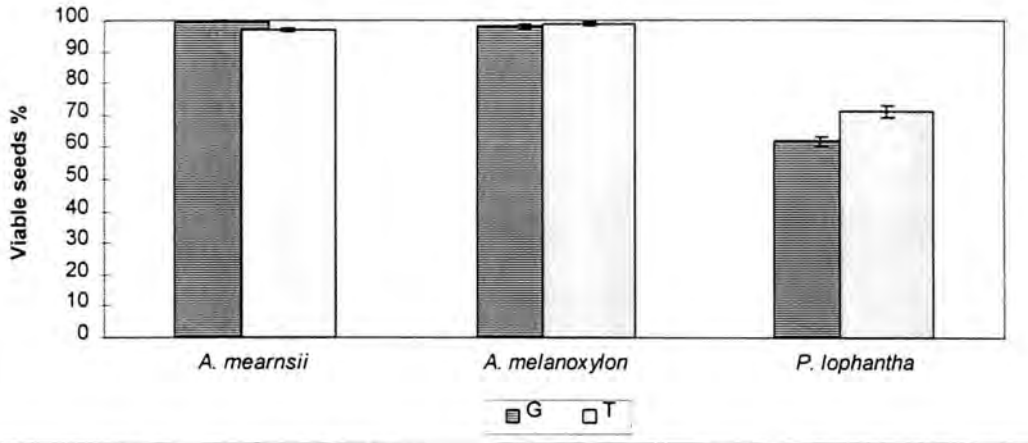

Figure 4 The viability of seeds of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* after storage of 8 months as determined by germination tests (G) and the tetrazolium chloride method (T). (Vertical bars indicate standard deviation).

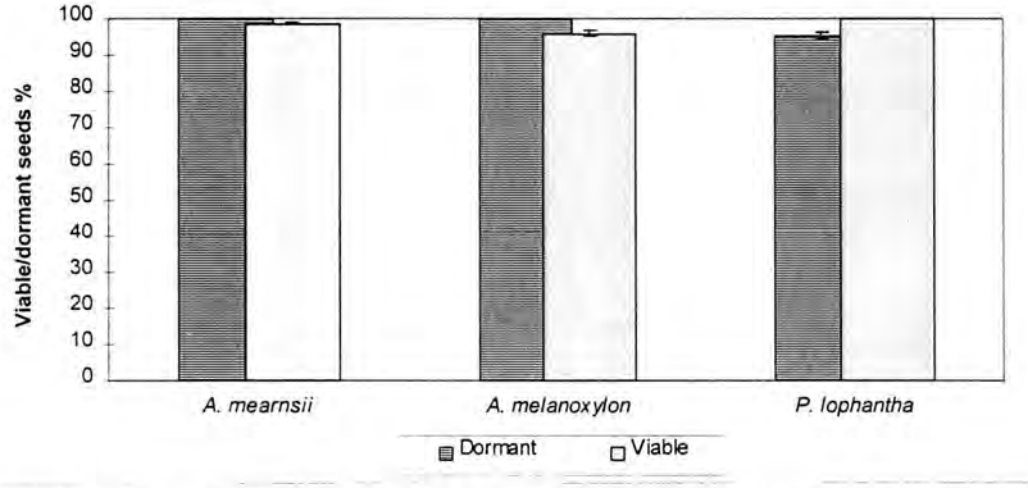

Figure 5 The dormancy (D) and viability (V) of fresh *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* seed. (Vertical bars indicate standard deviation).

Dormancy

P. lophantha seeds are slightly but significantly less dormant than the other two species (P=0.0086) which both have 100% dormant seeds (Figure 5).

Attractiveness to ants

In all four cafeteria experiments, *A. mearnsii* diaspores were significantly (P<0.001) more attractive to ants than either *A. melanoxylon* or *P. lophantha*. The mean percentage of remaining seeds over the four experiments, is shown for each ant nest in

Table 3 (There is more variation between ant nests than between the different experiments).

Table 3 The mean number of remaining diaspores in four cafeteria experiments where 10 diaspores of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha* were presented to ants next to nest entrances. (± indicate S.E. The mean percentages of four different experiments are presented because there was almost no variation between the results of the experiments).

		Plant species		
Nest number	Ant species	*A. mearnsii*	*A. melanoxylon*	*P. lophantha*
1	*Tetramorium solidum* (Emery)	1.4 ± 2.6	9.6 ± 0.9	10 ± 0
2	*Ocymyrmex barbiger* (Emery)	2.2 ± 3.3	9.2 ± 0.8	10 ± 0
3	*T. quadrispinosum* (Emery)	0.6 ± 1.3	10 ± 0	10 ± 0
4	*Linepithema humile* (Mayr)	3 ± 2.5	9.8 ± 0.4	10 ± 0
5	*T. quadrispinosum* (Emery)	0 ± 0	9 ± 1.0	10 ± 0
6	*T. quadrispinosum* (Emery)	1.4 ± 1.7	8.8 ± 1.8	10 ± 0

A. mearnsii diaspores were removed to a large extent, while only a few *A. melanoxylon* diaspores and no *P. lophantha* diaspores at all were removed. This trend, as well as the rate of removal of *A. mearnsii* seed, is shown in Figure 6 a,b. No ants were observed to remove arils or part thereof of any species, instead, whole seeds were taken into the nest entrance. The only seeds without arils that were found on the third day of the experiments, were *A. mearnsii* seeds that had probably been taken into the nest, the aril removed and the seeds ejected onto the middens. At nests 1, 2 and 4, no seeds were ejected, while at nests 3, 5 and 6 respectively, 59, 15 and 16 seeds without arils were ejected from the nests.

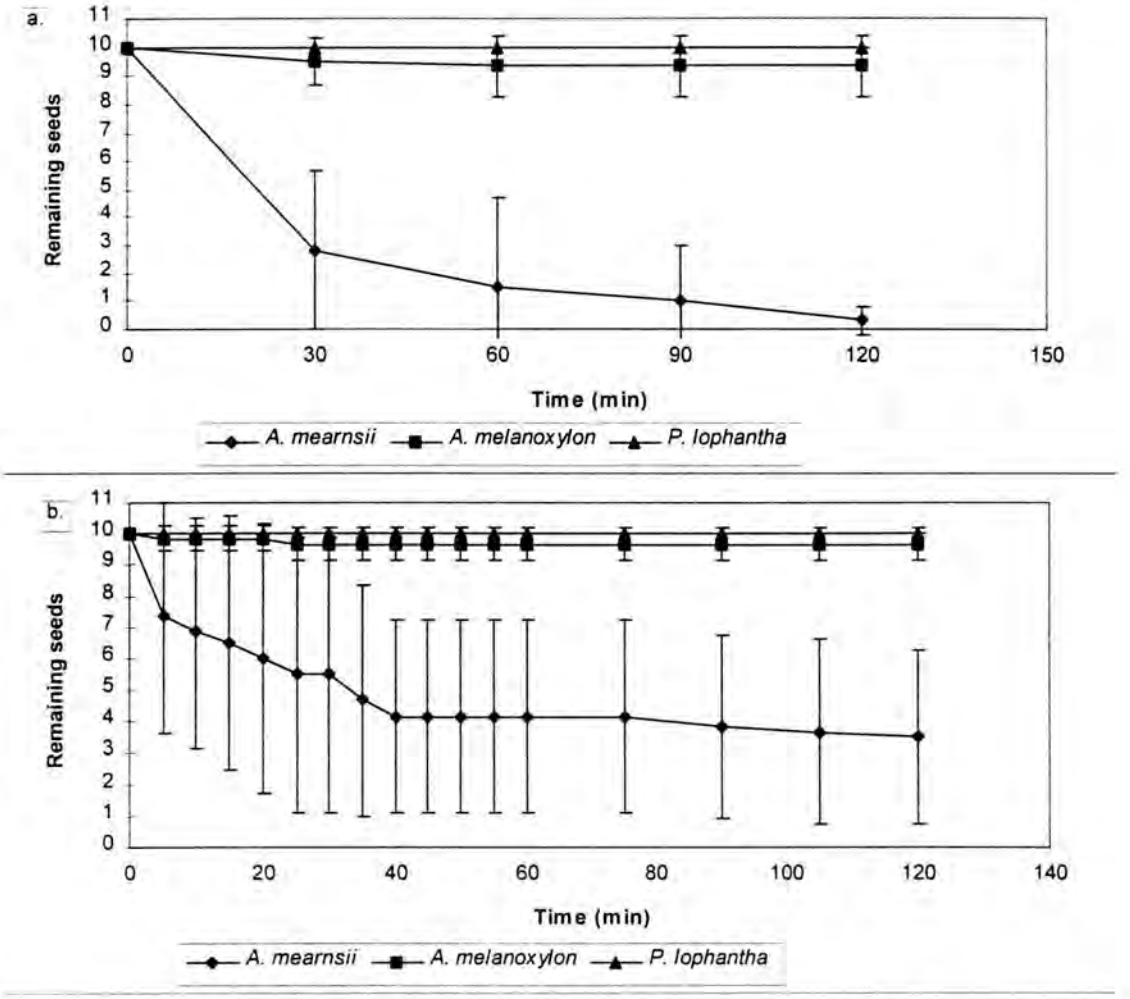

Figure 6 The mean rate of removal of diaspores of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha* that was presented to ants next to six different nest entrances on: **a.** Day 1 and **b.** Day 2. (Vertical bars indicate standard deviation).

2.4 Discussion

Aril colour is not an absolute characteristic for dispersal syndrome in *Acacia*, but is probably the best single discriminator between ant- and bird-dispersed species (Davidson & Morton 1984; O' Dowd & Gill 1986). *A. melanoxylon* with its dull red/pink aril colour in South Africa therefore appears to fall into the bird-dispersal category while *A. mearnsii* and *P. lophantha* with their smaller, respective off-white and brownish arils would fall into the ant-dispersal category (Figure 1). Published data on other parameters such as aril mass, and percentage investment in dispersal (dry aril mass/wet diaspore mass ratio) (O' Dowd & Gill 1986) support the

presumption that *A. melanoxylon* is bird-dispersed and the other two species are ant-dispersed (Table 1). Aril mass (4.63 mg) and percentage investment in dispersal (20.48%) of *A. melanoxylon* in South Africa corresponds roughly to the values found in Australia (3.9 mg and 28.7% respectively) (O' Dowd & Gill 1986). Aril mass and percentage investment in dispersal of *A. mearnsii* in South Africa (0.8 mg and 5.07%) also corresponds with the Australian values (0.8 mg and 5.1%) found by O' Dowd and Gill (1986). *A. melanoxylon* also displays diaspores in the dehiscent pods for an extended period of time, another characteristic indicating adaptation for bird-dispersal. The diaspores of *A. mearnsii*, on the other hand, are presented on the tree for a relatively short time before they drop either before or together with the opened seed pod. No comparative data for *P. lophantha* in Australia could be found in the literature. The parameters used to distinguish between ant- and bird-dispersed *Acacia* species in Australia (Davidson & Morton 1984; O' Dowd & Gill 1986) will not necessarily be applicable to *P. lophantha*, being a different species. However, *P. lophantha* is a member of the Mimosoideae, was previously classified under *Acacia* (Nielsen *et al.* 1983a) and is classified as an ant-dispersed species by Pemberton and Irving (1990). It might therefore be justifiable to assess *P. lophantha* seed according to the abovementioned parameters for *Acacia*. *P. lophantha* has a relatively large seed with a small aril (0.35 mg) and therefore the percentage investment in dispersal is very small (0.51%). Although the aril contains elaiosomes (Pemberton & Irving 1990), the small investment made in dispersal structures suggest that *P. lophantha* might not be primarily adapted to ant- or bird-dispersal. *P. lophantha* diaspores also drop either from the seed pods or along with the seed pods. However, observations in a quiet, wind-protected valley showed that opened *P. lophantha* pods with seeds still inside, were present on the trees 4-5 months after they ripened. The cafeteria experiments support the hypothesis that *P. lophantha* is not a mymecochore in the South Western Cape. Ants showed mild interest in *A. melanoxylon* diaspores but rarely remove them while *A. mearnsii* diaspores are removed rapidly (Figure 6a,b). Therefore, in South Africa, *A. melanoxylon* can be classified as an ornithochore, *A. mearnsii* as a myrmecochore while *P. lophantha* is not adapted to either ant- or bird dispersal. The large papery pods of *P. lophantha* that tend to drop with the diaspores

still inside them, may be an adaptation for hydrochory. All three species were listed as being water-dispersed by Richardson *et al.* (1992).

The ecological significance of seed size has been extensively investigated by, amongst others, Salisbury (1942), Baker (1972) and Fenner (1983). The general theory accepted by these authors is that bigger seeds are produced by plants whose seedlings are prone to being overshadowed and/or woody plants of later successional stages and plants from a forest environment. Paradoxically, bigger seeds are also found in plants growing under severe xeric conditions. Large seeded species are able to establish more successfully in the shade (Grime & Jeffrey 1965) while their seedlings have a growth advantage over small seeded species only under shady conditions (Gross 1984). The reason is that large seeds have bigger energy reserves than small seeds (Salisbury 1942). *P. lophantha*, having a significantly bigger seed than the other two species (Table 1), therefore appears to be better adapted to establishing itself under and growing in shady conditions. Based on seed size alone, *A. mearnsii* and *A. melanoxylon* should have the same ability to establish and grow under shady conditions. However, seed size is not the only criterion available to predict the probability of a seedling establishing and growing in a particular environment. Chemical composition of seeds also influences the capability of a seedling to establish itself under adverse conditions (Levin 1974). Oil, carbohydrates and proteins are the main sources of energy reserves in seeds and, of these three components, oil is the most important, because of its high relative energy content (Levin 1974). As with seed size, oil content of seeds also increases with the increased woodiness of plant species. Fenner (1983) found that bigger seeds in the Asteraceae had a higher carbon, but lower ash content. This is an indication that the establishment of seedlings from big seeds is usually limited by shading, while that of small seeds is limited by nutrients. *P. lophantha*, producing seeds 3-4 times bigger than the other two species, does not have a higher carbon content or lower ash content than the other two species. *A. melanoxylon*, on the other hand, has a significantly higher crude protein and ash content than *A. mearnsii*, which is about the same size (Table 1). When individual elements are taken into consideration, it is clear that *A. melanoxylon* has the highest content of the most important elements. *A. mearnsii*, however has almost double the oil content of the other two species. Although the

occurrence of ant-dispersed seeds correlates positively with areas of low fertility and the occurrence of bird-dispersed species correlates positively with more fertile environments (Milewski & Bond 1982), the bigger ash and individual mineral content of *A. melanoxylon* seeds indicates that it would be more in need of mineral nutrients in the early life stages. Being bird-dispersed, seeds whose dormancy might be broken by passage through the gut of a bird, would be deposited mostly under bird perches, and would probably have to establish under existing vegetation. Normally areas under trees are more fertile than areas in the open, and the high mineral content of the seeds is therefore an anomaly. According to Davidson and Morton (1984) the higher mineral content (especially N) under tree canopies in the Australian arid zone, might be partly due to kangaroos sheltering under the trees. In more mesic forests, such as those where *A. melanoxylon* occurs (Costermans 1981), there would probably not be such a concentration of animals, and therefore a lower mineral nutrient content should prevail. *A. mearnsii* on the other hand, being ant-dispersed, is probably deposited in or around ant nests which are also higher in nutrient content than the surrounding vegetation (Berg 1981). *A. mearnsii* would therefore not need such a high nutrient reserve, but the burial of seed by ants, makes it imperative to have a high energy reserve to enable the seedlings to emerge from deeper germination depths than for instance *A. melanoxylon*, which will probably be deposited on the soil surface by birds. Such an explanation might explain the higher oil content of *A. mearnsii*. *P. lophantha* shows no obviously high content of any of the substances. If indications that *P. lophantha* is not primarily ant dispersed are correct, it will also remain on or near to the soil surface and would have no need to establish from great depths, except when buried in loose alluvial deposits by water action.

The testa surfaces of the three species does not conform to the general assumption that mimosoid seeds with thick cuticles (10 μm or more) have smooth surfaces (Van Staden *et al.* 1989). Both *A. mearnsii* and *P. lophantha* have cuticles thicker than 20 μm (Table 2) and both show a sculptured cuticle (Figure 3d,f). All three species display pleurograms that extend completely through the epidermis (Figure 3g,h,i). The cuticle, palisade layer and light line have been implicated as causing water impermeability in various seeds (Rolston 1978; Werker 1980/81). In the case of these three species, it is doubtful if any of these structures can be responsible for water

impermeability, as the pleurograms extend right through these structures to the parenchyma layer, similar to the pleurogram of *Albizia adianthifolia* (Van Staden *et al.* 1989). Tran and Cavanagh (1980) found that impermeability of *Acacia farnesiana* seeds could only be broken if holes extended past the boundary between palisade and hour glass cells. This implies that impermeability of the seed coat is located at least partly in the lower regions of the palisade layer. In *P. lophantha*, this might be due to the thick layer of PAS positive material at the base of the palisade layer (Dell 1980). The layer was present in *P. lophantha* but could not be distinguished in the other two species (Figure 3g,h,i).

Water uptake by legume seed after heat treatments occurs via the strophiole (lens) (Tran 1979; Dell 1980; Hanna 1984; Manning & Van Staden 1987; Van Staden *et al.* 1989). The testa, therefore, does not seem to play a role in the softening of the seeds by heat treatments. Seed testa structure and thickness, however, do play a role in the natural softening of seeds (Morrison *et al.* 1992). Where dormancy is broken by acid treatment, the lens also plays a role in allowing water imbibition, but other areas of the seed testa also allows water into the seed (Manning & Van Staden 1987). Kelley and Van Staden (1985) showed that acid scarification caused weak spots in the seed testa of *Aspalathus linearis* to allow imbibition to take place. It therefore appears as if the structure of the seed testa, apart from the area of the lens, may play a role in the natural softening of hard seeds with time, and in acid scarification, but not in heat treatments. Acid scarification generally influences the palisade cells and if the thickness of the palisade layer could be an indication of the sensitiveness for acid scarification, *P. lophantha* should be the least susceptible to acid treatments, followed by *A. mearnsii* and then by *A. melanoxylon* (Table 2). If *A. melanoxylon* is indeed more susceptible to acid treatments, the adaptation for bird dispersal could also be an adaptation for the breaking of dormancy too, since Pieterse (1986) found that *A. longifolia* seeds that passed through the gut of redwing starlings (*Onychognathus morio morio*) had a dormant proportion of 60%, compared to the 94% of seeds fresh from the tree. Ingestion by birds might therefore reduce the dormancy of *Acacia* seeds.

Morrison *et al.* (1992) found that loss of seed dormancy with time is related to the structure of the seed testa. *Acacia* species that lost their dormancy naturally generally had a thinner testa, with a relatively thicker palisade layer (61.3% of the total testa thickness) and a relatively thinner parenchyma layer (24.5% of the total testa thickness). None of the three species investigated had a similar composition of the seed testa, although *A. melanoxylon* had a relatively thinner palisade layer and thicker mesophyl layer than the other two species. This could be an indication that *A. melanoxylon* does not lose its dormancy quickly.

The relatively low viability of the eight month old *P. lophantha* seed (Figure 4) was probably a result of the seed being picked too early *i.e.* with a high moisture content and then being stored in an airtight plastic container. Fungal or bacterial attack caused the non-dormant (wet) portion of the seeds to lose their viability. In the next experiment, where seeds that were dry enough and already dormant, were picked, the viability of the seeds was 100%. Dormancy (i.e. impermeability) of the seeds of all three species is high (>95%) (Figure 5).

The seed and seed testa characteristics of the three species investigated in South Africa appears to correspond with the available data from Australia. According to data from Australia, and data obtained in South Africa, *A. melanoxylon* can still be categorised as a bird-dispersed species, while *A. mearnsii* is adapted for ant-dispersal. *P. lophantha* however, is not a myrmecochore, probably because the percentage investment in dispersal is so small and the seed size so big (Table 1). Therefore hypothesis one is accepted.

Hypotheses two is refuted by the data. *A. melanoxylon* seeds differ markedly from the other two species in terms of testa composition. The relatively thin cuticle and palisade layer and the relatively thick mesophyl layer might be an adaptation for bird dispersal because it causes the seed to be better suited for acid scarification than heat damage to break seed dormancy. An extensive study of the seed testas of several bird-dispersed and ant-dispersed *Acacia* species is however needed to confirm the indications in this study that such a pattern exists.

Chapter 3

Germination characteristics of three woody legume invaders

3.1 Introduction

Distribution of plants is controlled by the environment and the adaptation of the plants to environmental conditions (Harper 1977). The first obstacle that a plant has to overcome to establish itself in a particular area, is for the seed to find a "safe site" (Harper 1977). However, the safety of a site depends on the conditions that occur there during its establishment phase after the seed has germinated. The seed can only respond to existing conditions, so natural selection has presumably resulted in each species responding to a combination of factors for germination which have a high probability of being followed by another combination of factors favourable for establishment (Fenner 1985). Thus, the seeds requirements for germination are generally an indication of the environment where the plants will survive.

Factors influencing germination of seeds are temperature, moisture, light, micro-atmosphere around the seed, chemical substances, etc. (Mayer & Poljakoff-Mayber 1982). Any seed has temperature limits that inhibit germination and within these limits, an optimum temperature where optimal germination will occur, exists (Mayer & Poljakoff-Mayber 1982). Some seeds require fluctuating temperatures and will not germinate at any constant temperature (Thompson *et al.* 1977). Thompson *et al.* (1977) also observed that an interaction between light and temperature fluctuations is necessary to stimulate germination of some seeds. Light is another factor which is crucial for the germination of some seeds, while the germination of other seeds are light-independent (Smith 1973). The availability of moisture also plays a crucial role in seed germination (Simon 1984). The effect of artificial moisture stress on the germination of seeds has been investigated by germinating seeds in solutions containing varying concentrations of polyethylene glycol, mannitol or sodium chloride, which in turn creates varying osmotic potentials (Sharma 1973). Sharma (1973) however warned that osmotic solutions could not be used unconditionally to determine drought resistance of plants, because factors such as particle size and soil

water conductivity also play a role. The extent to which germinating seeds exhibit drought resistance, is therefore not necessarily an indication of the drought resistance of the adult plants. The effects of hydration-dehydration cycles on seed viability and germination, are also important, as they simulate conditions in nature under which seeds have to germinate (Fenner 1985). Chemical substrates such as nitrates and nitrites, *inter alia,* also influence the germination of seeds (Popay & Roberts 1970).

Most leguminous seeds are dormant because of a water impermeable seed coat (Rolston 1978; Werker 1980/81; Van Staden *et al.* 1989). Once the seed coat has been damaged and imbibition takes place, most legume seeds germinate readily. Factors such as temperature, light and moisture might however influence subsequent germination and these factors will be investigated in this study. The study might shed light on factors limiting the distribution of *Acacia mearnsii* De Wild., *Acacia melanoxylon* R. Br. and *Paraserianthes lophantha* (Willd.) Benth.. The following hypothesis is tested *viz.*: *A. melanoxylon*, originating from a less open habitat, will be less tolerant to extreme germinating conditions than the other two species which occur in more open habitat in their country of origin.

3.2 Materials and methods

Ripe seeds were harvested from *A. mearnsii, A. melanoxylon* and *P. lophantha* trees in the vicinity of Stellenbosch (33° 56' S; 18° 52' E) in December 1989 and were stored for about six months in glass jars under dry, dark conditions before being used. Seeds were again harvested from the same localities in December 1990 and December 1991.

Method of inspection

A germination experiment was executed to determine whether different methods of inspection, such as repeated opening of petri-dishes (*e.g.* to determine rate of germination under light conditions) or opening petri-dishes once at the termination of the experiment (*e.g.* to determine germination percentage under dark conditions) would influence seed germination. Possible variation in germination may occur due to variable oxygen levels as a result of respiration by the imbibing and germinating seeds (Bewley & Black 1994). In a petri-dish that stays sealed for several days oxygen levels might be lower than in a petri-dish that is opened daily.

Two methods of inspection *viz.* 1) repeated inspection of one petri-dish for the duration of the trial and 2) a different petri-dish for each inspection time, were compared. This was done in order to determine whether the number of petri-dishes and seed needed in future comparisons between light and dark treatments could be minimized. Seeds of the three species were chipped at the distal end to break dormancy. The seeds were immediately transferred into 90 mm plastic petri-dishes containing two filter paper discs and 6 ml of a benomyl\captab solution (0.022% and 0.055% m/v a.i. respectively) following Clemens *et al.* (1977). The petri-dishes were placed in polyethylene bags that were sealed to prevent moisture loss. The bags containing the petri-dishes were incubated in a growth chamber under dark and light conditions at a constant temperature of 20° C. Petri-dishes were divided into two groups which were both kept under light and dark conditions. Light was provided by florescent tubes and a 100 w incandescent light bulb resulting in a light intensity of 25.2 $\mu mol\ s^{-1}\ m^{-1}$. The light source was similar for all other experiments where light was provided. Petri-dishes receiving the dark treatment were wrapped in aluminium foil before being placed into the polyethylene bags. Inspections for germinated seeds were done after 4, 8, 12 and 16 days using one of two methods. The petri-dishes in one group were kept closed until opened for inspection. The 16 days treatment, for example, was opened for the first time after sixteen days. All the petri-dishes in the second group were opened at every inspection date and germinated seeds were removed from them. The 16 days treatment would therefore have been opened and closed on three previous occasions. The germination percentage for each treatment was determined.

Effect of fungicide and light on germination

A germination test was executed to determine 1) the effect of a captab/benomyl mixture on germination and 2) the effect of light and dark conditions on germination. This was done to determine whether the captab/benomyl mixture added to curb fungal growth in germination experiments, influenced seed germination adversely under light or dark conditions. Seeds of the three species were chipped at the distal end to break dormancy. Sixteen replicates of 25 seeds each were used in a 3x2x2 factorial design. The three species were subjected to two fungicide treatments i.e. with and without a

captab/benomyl mixture (0.022% benomyl and 0.055% captab m/v (a.i.) respectively) under light and dark conditions. Seeds were put into petri-dishes as described earlier. Six ml of the various solutions were added to the petri-dish. The petri-dishes were incubated as in previous experiments. Petri-dishes receiving the dark treatment were wrapped in aluminium foil before being placed in the polyethylene bags.

Four replicates of each treatment combination were opened and inspected after 4, 8, 12 and 16 days respectively. At each inspection the number of seeds which had germinated (i.e. radicle protruding more than 1 mm) were recorded and germination percentages were calculated.

Effect of light and temperature

A pilot study was undertaken to determine if an interaction between light and temperature existed. In this pilot study only three temperatures were investigated. Four replications of 25 seeds of each species were treated as follows: Seeds were chipped at the distal end and germinated under light and dark conditions as described above. Germination temperatures were kept constant at 10, 20 and 30°C respectively. Petri-dishes were opened after 8 days when the germinated seeds were counted and the germination percentages were calculated.

Effect of constant temperature

Four replications of 25 seeds of each species were treated as follows: Seeds were chipped and incubated under dark conditions as described above under different constant temperature regimes in growth cabinets. Treatments were at constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40°C respectively. Seeds were inspected for germination daily for 14 days. The viability of ungerminated seeds that did not rot, was determined by means of tetrazolium staining (Moore, 1973). Germination percentages were calculated and the germination rate of treatments were calculated using the following equation (Heydecker 1973):

$$CRG = \frac{\sum n}{\sum (D.n)} \cdot \quad 100$$

where CRG is germination rate, n equals the number of seeds germinating on day D and D equals number of days, counted from the day of sowing, which is 0.

Effect of alternating temperatures

Four replications of 25 seeds per species were treated as follows: Seeds were chipped and incubated as described before under alternating light and dark conditions. The seeds were subjected to 12/12 hour day/night temperature regimes of 15/20°C , 15/25°C, 15/30°C, 20/25°C, 20/30°C and 20/35°C. Seeds were inspected for germination daily for 14 days. Ungerminated seeds that did not rot, were tested for viability by means of the tetrazolium method (Moore 1973). Percentage germination and germination rate were calculated for each treatment.

Rate of imbibition and germination

The rate of imbibition of twelve seeds of each species was investigated to determine if any species would have an advantage of faster germination due to rate of imbibition. Each seed was chipped at the distal end with a sharp blade, weighed and then transferred to a glass vial containing 10 ml of water at 20°C. The vials containing the water and seed were then incubated at 20°C under continuous light. The seeds were removed from the vial, blotted dry and weighed every six hours for 24 hours. Each seed was then transferred to individual petri-dishes containing two Whatman No. 1 filter papers and 6 ml of a cabtab/benomyl mixture as described earlier. Each seed was weighed after 48 and 72 hours respectively, when germination started. The mean mass, mass increase and relative mass increase over the incubation period was calculated.

Another experiment similar to the one above were executed, with the difference that the seeds were weighed hourly for 32 hours. Rate of imbibition of the three species was compared by determining the mean time required to reach 95% of the final mass. The significance of the difference between the means of this parameter was determined by an ANOVA analysis (Snedecor & Cochran 1980).

Effect of varying periods of imbibition and desiccation

Four replications of 25 seeds of each species were treated as follows: Seeds were chipped at the distal end and allowed to imbibe water by immersion into distilled water kept at a constant temperature of 20°C, for periods of 0, 3, 6, 12 and 24 hours. After the imbibition period, seeds were dried and stored at room temperature in open,

plastic petri-dishes for periods of 0, 1, 3, 7 and 14 days. After that, seeds were placed in petri-dishes in the normal way and then incubated to germinate at a constant temperature of 20°C in the dark, as described before. Seeds were inspected for germination after 7 days, and the viability of the remaining seeds were tested by means of the tetrazolium method. The emerging radicle of the germinated seeds were also measured to obtain an indication of the vigour of the seed after the treatment.

Effect of various solutes with varying osmotic potentials

Four replications of 25 seeds of each species were treated as follows: Seeds were chipped at the distal end and were incubated in petri-dishes containing different germination solutions. A range of concentrations of three different chemical compounds, mannitol, polyethylene glycol and sodium chloride were prepared to provide solutions of different osmotic potentials. Osmotic potential of the solutions was determined by means of a Wescor 3100 vapour pressure osmometer. Regression coefficients of more than 0.95 were obtained between osmotic potential and concentration of the solutions. From these calibrations, a range of solutions were prepared with osmotic potentials of 0, -0.5, -1, -1.5 and -2 MPa. These solutions were used as germinating solutions, and the seeds were incubated at 20°C in the dark as described above, with the exception that the fungicide mixture was not added to the germination solution. It was thought that the addition of fungicide to the solutions would alter the osmotic potential of the solutions or that chemical reactions could have taken place. Germinating seeds were recorded every second day for 14 days. Percentage germination and germination rate were calculated for each treatment.

In all the germination tests the significance of differences between means of treatments were determined by using the LSMEANS command of PROC GLM (SAS Institute Inc., 1985). Germination percentage data were logit transformed before analysis.

3.3 Results

Method of inspection

There is a significant interaction (P=0.021) between method of inspection and time of inspection (Figure 1a). The percentage of germinated seeds is highest after 12 days

where petri-dishes are opened but highest after 8 days where petri-dishes are kept closed until time of inspection. The fact that there are no significant differences in percentage germination after 16 days, indicates that the differences after 8 days and after 12 days are possibly of no biological significance. A significant interaction (P=0.0302) also exists between species and method of inspection where *A. mearnsii* and *P. lophantha* have higher germination percentages where the petri-dishes are opened but *A. melanoxylon* has similar germination percentages for each method of inspection (Figure 1b). Light has no significant (P=0.7580) effect on germination percentage of any of the three species. The differences are so small that it is concluded that the opening or not of the petri-dishes has no real effect and that in future experiments only one set of light treatment petri-dishes is necessary even if multiple counts are made.

Effect of fungicide and light on germination

There is no significant interaction (P=0.9923) between species, light, fungicide and time of inspection. A significant interaction (P=0.038) between species, light and fungicide exists. Addition of fungicide decreases the difference in germination percentage between dark and light conditions for *A. mearnsii* and *A. melanoxylon*, but not for *P. lophantha* (Figure 2). More fungal growth occurs under light conditions and the fungicide inhibits fungal growth, resulting in even germination percentages under light and dark conditions. Significantly more seed of all species germinate after 8 days than after 4, 12 and 16 days (Figure 3). This could be the result of more seeds dying due to fungal attack after 12 and 16 days but again the differences are so small that they are of no biological significance.

Effect of light and temperature

The only significant interaction occurs between species and temperature (P=0.0059). *A. melanoxylon* does not germinate at 10°C either in the presence or absence of light whereas *A. mearnsii* and *P. lophantha* achieve germination percentages of 80 and 90% respectively (Figure 4). Light has no effect on germination of any of the species (P=0.3751).

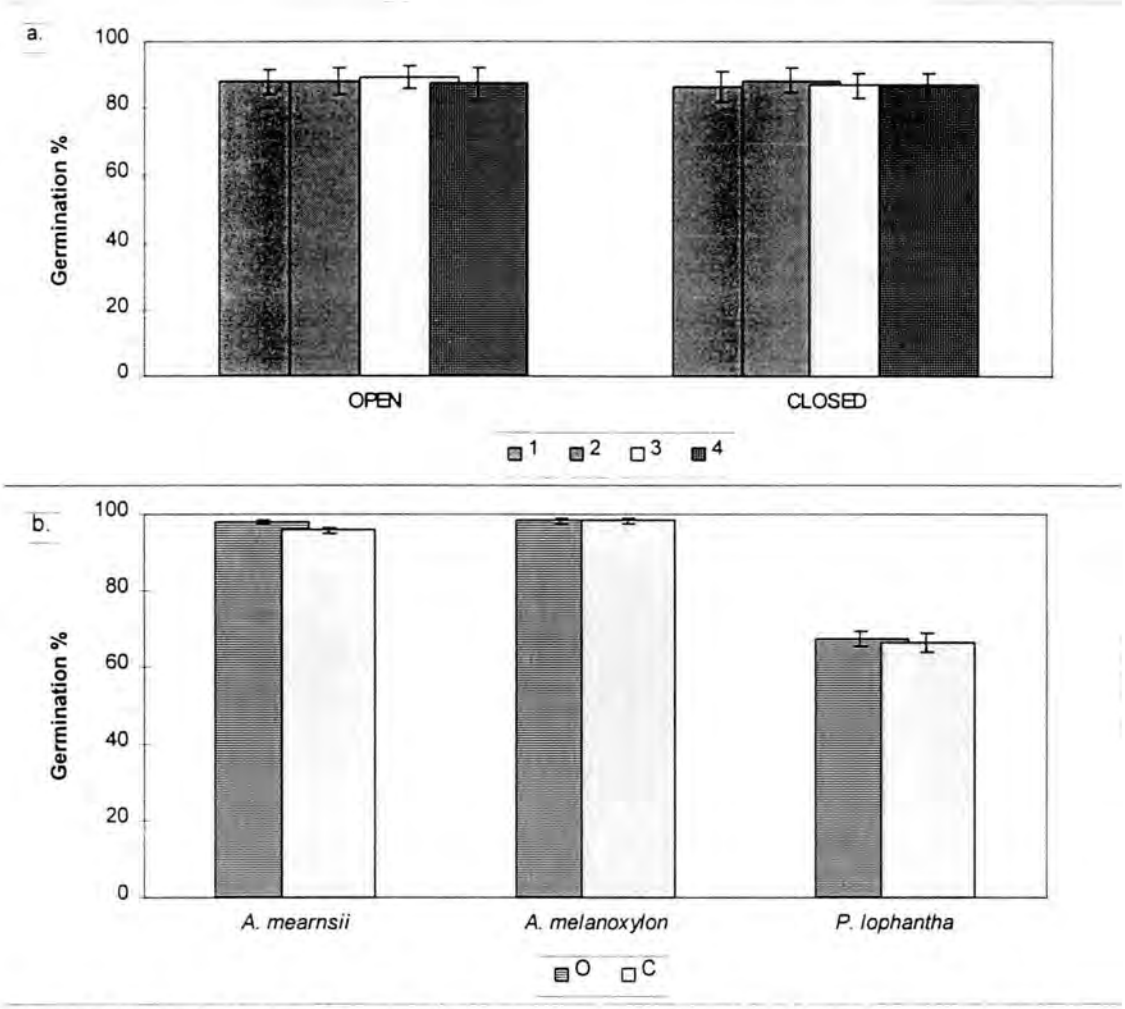

Figure 1 **a.** The influence of inspection method (open and closed) and time of incubation (1=4 days, 2=8 days, 3=12 days and 4=16 days) on the germination of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* seeds. **b.** The influence of inspection method (O = open and C = closed) on germination of *A. mearnsii*, *A. melanoxylon* and *P. lophantha* after 16 days of incubation. (Vertical bars indicate standard deviation).

Effect of constant temperature

A significant (P=0.001) interaction occurs between species and temperature when germination percentage is considered. *A. melanoxylon* is negatively influenced by the lowest and highest temperatures while *A. mearnsii* is negatively influenced only by the highest temperatures (Figure 5a). *P. lophantha* is the least influenced by the range of temperatures.

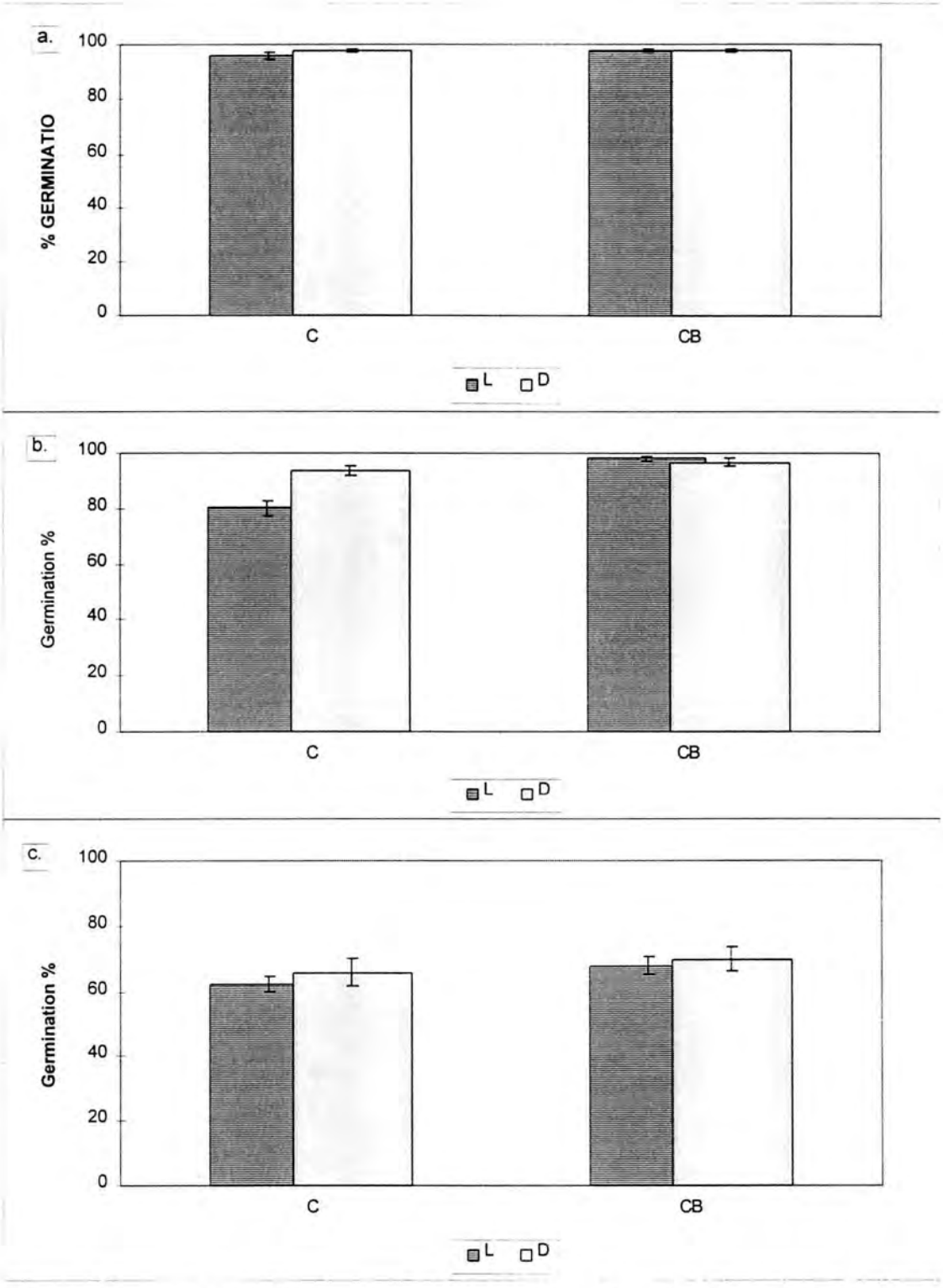

Figure 2 The germination of **a**. *Acacia mearnsii*, **b**. *A. melanoxylon* and **c**. *Paraserianthes lophantha* seeds under light (L) and dark (D) conditions in the absence (C) and presence (CB) of a captab/benomyl mixture. (Vertical bars indicate standard deviation)

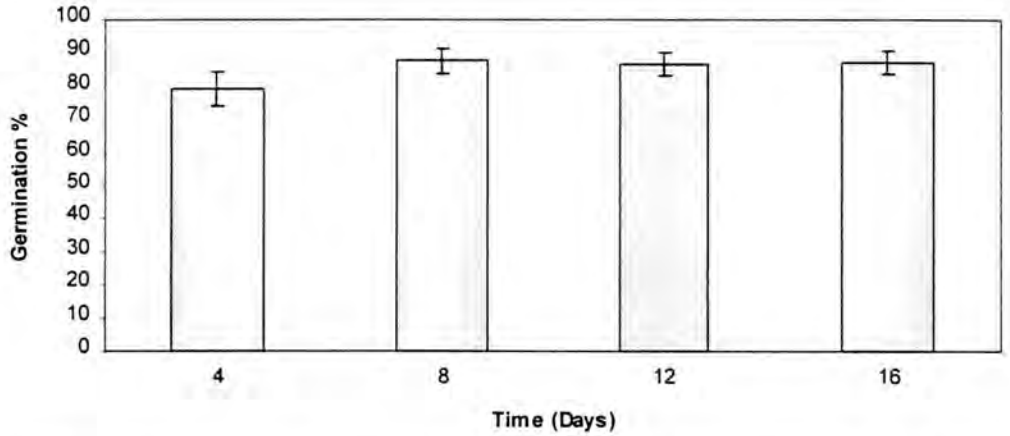

Figure 3 The germination percentage of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* seeds after 4, 8, 12 and 16 days of incubation. (Vertical bars indicate standard deviation).

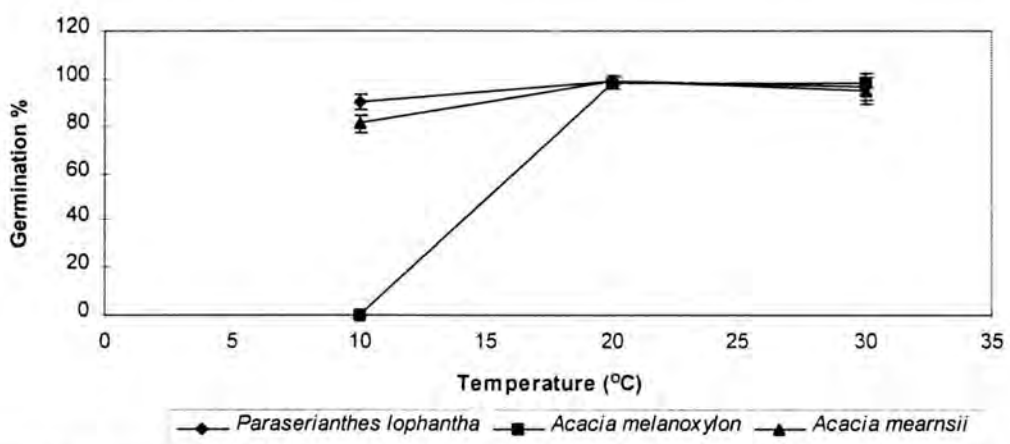

Figure 4 The interaction between temperature and species in the effect of light and temperature on the germination of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. (Vertical bars indicate standard deviation)

Regarding germination rate, a significant interaction (P=0.0001) occurs between species and temperature. Maximum germination rates for *P. lophantha* and *A. melanoxylon* occur at 25°C, while the maximum germination rate for *A. mearnsii* occurs at 30°C (Figure 5b). Considering both germination percentage and germination rate, it appears as if the optimum temperature for *A. melanoxylon* and *A. mearnsii* is 20 - 30°C, and for *P. lophantha* is 25 - 35°C.

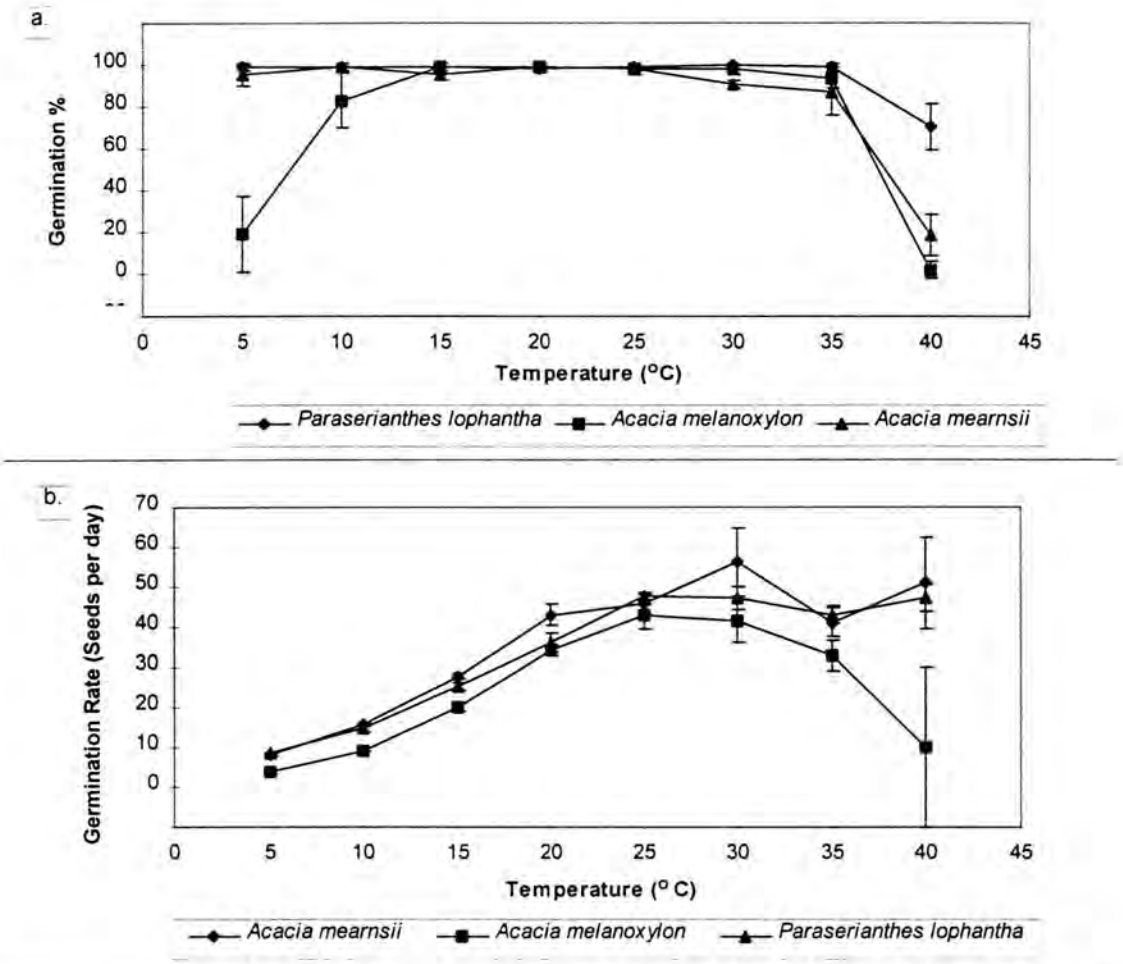

Figure 5 a. The effect of different constant temperatures on the germination percentage of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* seed. b. The effect of different constant temperatures on the germination rate of *A. mearnsii*, *A. melanoxylon* and *P. lophantha* seed. (Vertical bars indicate standard deviation)

TABLE 1 The effect of different fluctuating temperatures on the germination percentage of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. (+ indicate the standard error).

TEMPERATURES	*A. mearnsii*	*A. melanoxylon*	*P. lophantha*
15/20 °C	99 ± 1.0	100 ± 0	100 ± 0
15/20 °C	100 ± 0	98 ± 1.12	82 ± 2.0
15/30 °C	71 ± 7.2	95 ± 3.8	54 ± 10.0
20/25 °C	98 ± 1.16	100 ± 0	81 ± 3.4
20/30 °C	91 ± 0.68	99 ± 1.0	98 ± 2.0
20/35 °C	69 ± 15.76	98 ± 1.12	64 ± 16.32

Effect of alternating temperatures

A significant interaction (P=0.001) occurs between species and temperature in terms of germination percentage. Both *A. mearnsii* and *P. lophantha* appear to be negatively influenced by treatments where fluctuations between night/day temperatures were 15°C, whereas *A. melanoxylon* is not influenced by any of the temperature treatments (Table 1).

TABLE 2 The effect of different fluctuating temperatures on the germination rate of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. (± indicate the standard error).

TEMPERATURES	*A. mearnsii*	*A. melanoxylon*	*P. lophantha*
15/20 °C	20.0 ± 0	19.8 ± 0.14	20.0 ± 0
15/25 °C	24.8 ± 0.18	24.6 ± 0.22	23.9 ± 0.42
15/30 °C	20.0 ± 0	20.0 ± 0	20.0 ± 0
20/25 °C	24.8 ± 0.25	24.8 ± 0.25	23.8 ± 0.51
20/30 °C	27.4 ± 1.99	31.7 ± 0.94	33.3 ± 0
20/35 °C	23.7 ± 2.05	31.5 ± 0.75	31.8 ± 0.88

The germination rate is significantly influenced by the interaction between species and temperature (P=0.0001). No clear pattern can be distinguished. It does however appear as if the effect of treatments on germination rate corresponds with the effect of the treatments on germination percentage (Table 2).

Rate of imbibition

A. mearnsii and *A. melanoxylon* imbibe water much faster than *P. lophantha* (Figure 6a). After 12 hours, imbibition in *A. mearnsii* ceases (Figure 6a,b) while it continues for a further 6 hours in *A. melanoxylon* and 12 hours in *P. lophantha*. After 48 hours the seed mass of *P. lophantha* starts to increase again, but this is probably due to the onset of germination (Figure 6a,b). The mean relative seed mass increase shows that *A. mearnsii* has the highest rate of water uptake, followed by *A. melanoxylon* and *P. lophantha* (Figure 6c). This trend is confirmed in Experiment 2 where imbibition is measured at hourly intervals for 32 hours (Figure 7a,b,c). The ANOVA analysis, however, shows no significant difference in the time taken to reach 95% of the final

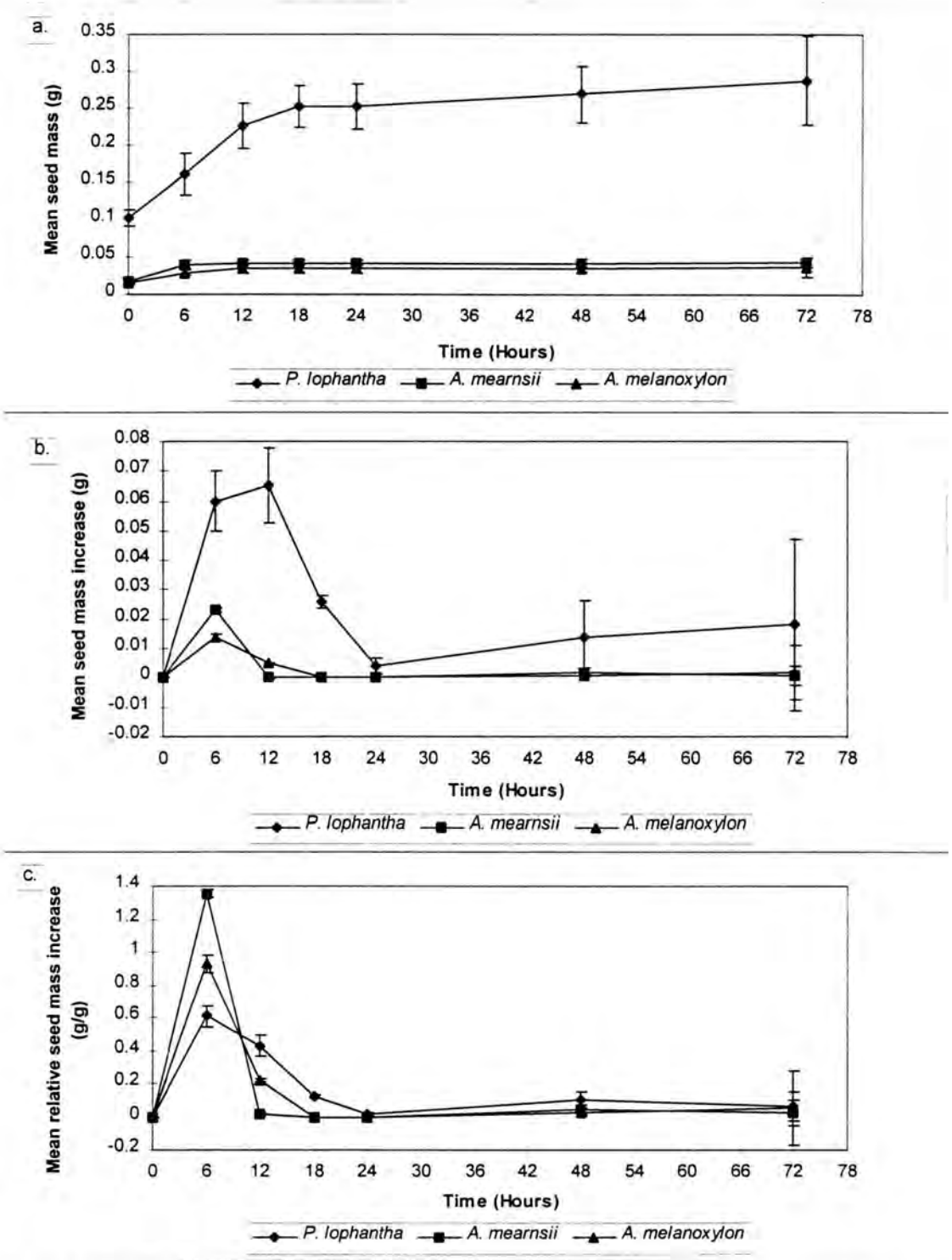

Figure 6 Imbibition rate of seeds of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha* measured at 6 hour intervals for 24 hours and thereafter every 24 hours up to 72 hours. **a.** Mean seed mass of the species with time of imbibition. **b.** Mean seed mass increase with time of imbibition. **c.** Mean relative seed mass increase with time of imbibition. (Vertical bars indicate standard deviation).

mass for *A. mearnsii* and *A. melanoxylon* (Table 3). *P. lophantha* takes significantly longer to reach 95% of the final mass (P=0.0083).

TABLE 3 Time taken for seeds of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha* to reach 95% of the final mass during imbibition (± indicate the standard error. Figures followed by the same letter do not differ significantly at the 5% level).

SPECIES	TIME (h)
Acacia mearnsii	5.88 ± 1.49 a
Acacia melanoxylon	7.33 ± 0.66 a
Paraserianthes lophantha	17.5 ± 0.45 b

Effect of varying periods of imbibition and desiccation

No significant interaction (P=0.0719) regarding germination percentage occurs between species, desiccation period and imbibition period. Significant interactions, however, occur between desiccation period and imbibition period (P=0.0001), species and imbibition period (P=0.0001) and species and desiccation period (P=0.0002). Seeds that receive no imbibition treatment, are not influenced by any period of desiccation. Seeds that are imbibed appear to germinate better after one day's desiccation. Seeds that are imbibed for 6 and 12 hours, are negatively influenced by 3 days, but not by longer periods of desiccation (Figure 8a). *P. lophantha* appears to be negatively influenced by imbibition treatments, especially the 6 hours imbibition period, whereas the *Acacia* species are not influenced by imbibition treatments (Figure 8b). *P. lophantha* seeds germinate better after a desiccation period of one day than when there is no desiccation period. The two *Acacia* species are not influenced by desiccation (Figure 8c). It therefore appears as if most of the variation causing the interaction between desiccation period and imbibition period (Figure 8a) can be attributed to *P. lophantha*.

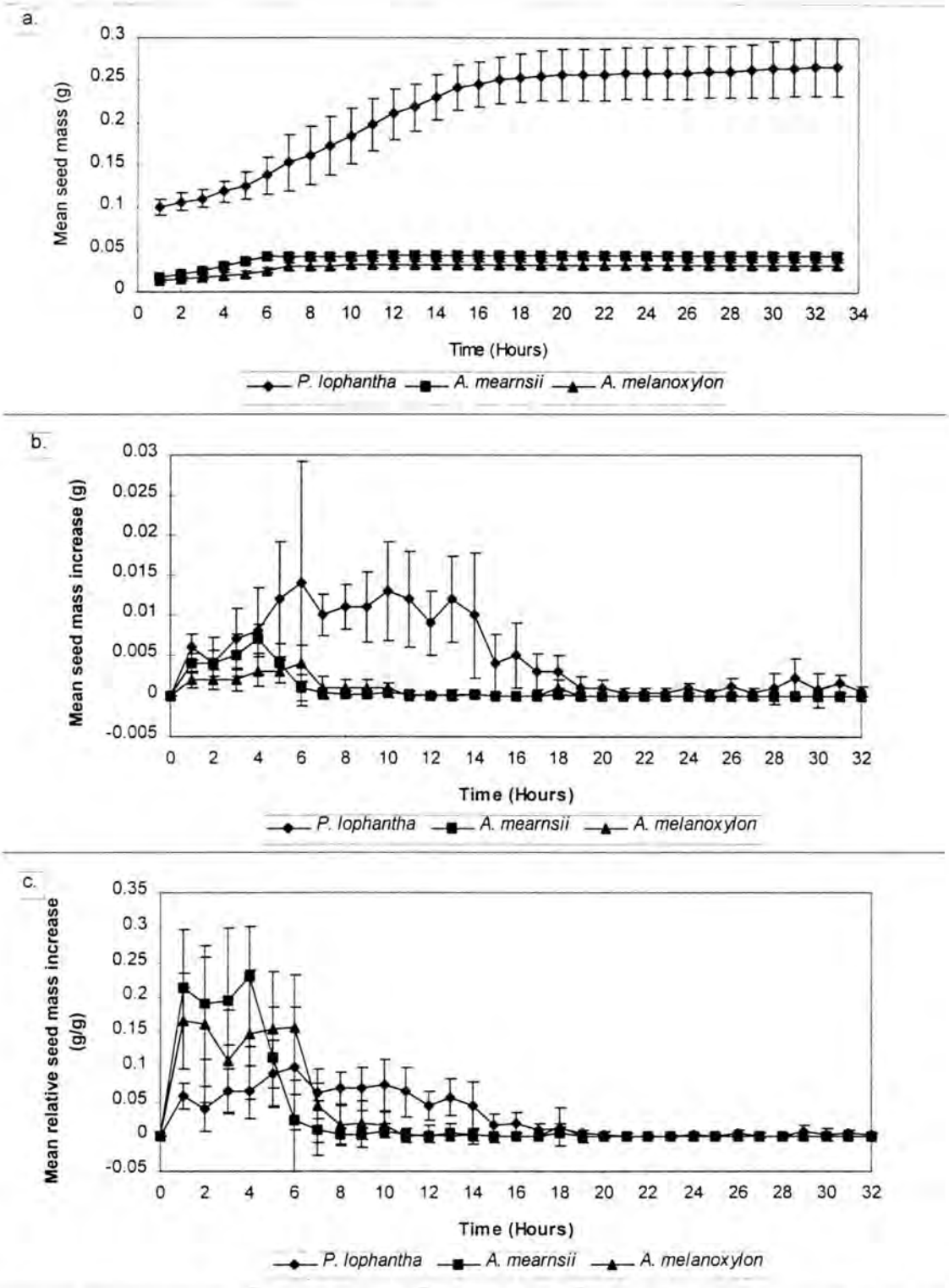

Figure 7 Imbibition rate of seeds of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha* measured at hourly intervals for 32 hours. **a**. Mean seed mass of the species with time of imbibition. **b**. Mean seed mass increase with time of imbibition. **c**. Mean relative seed mass increase with time of imbibition. (Vertical bars indicate standard deviation).

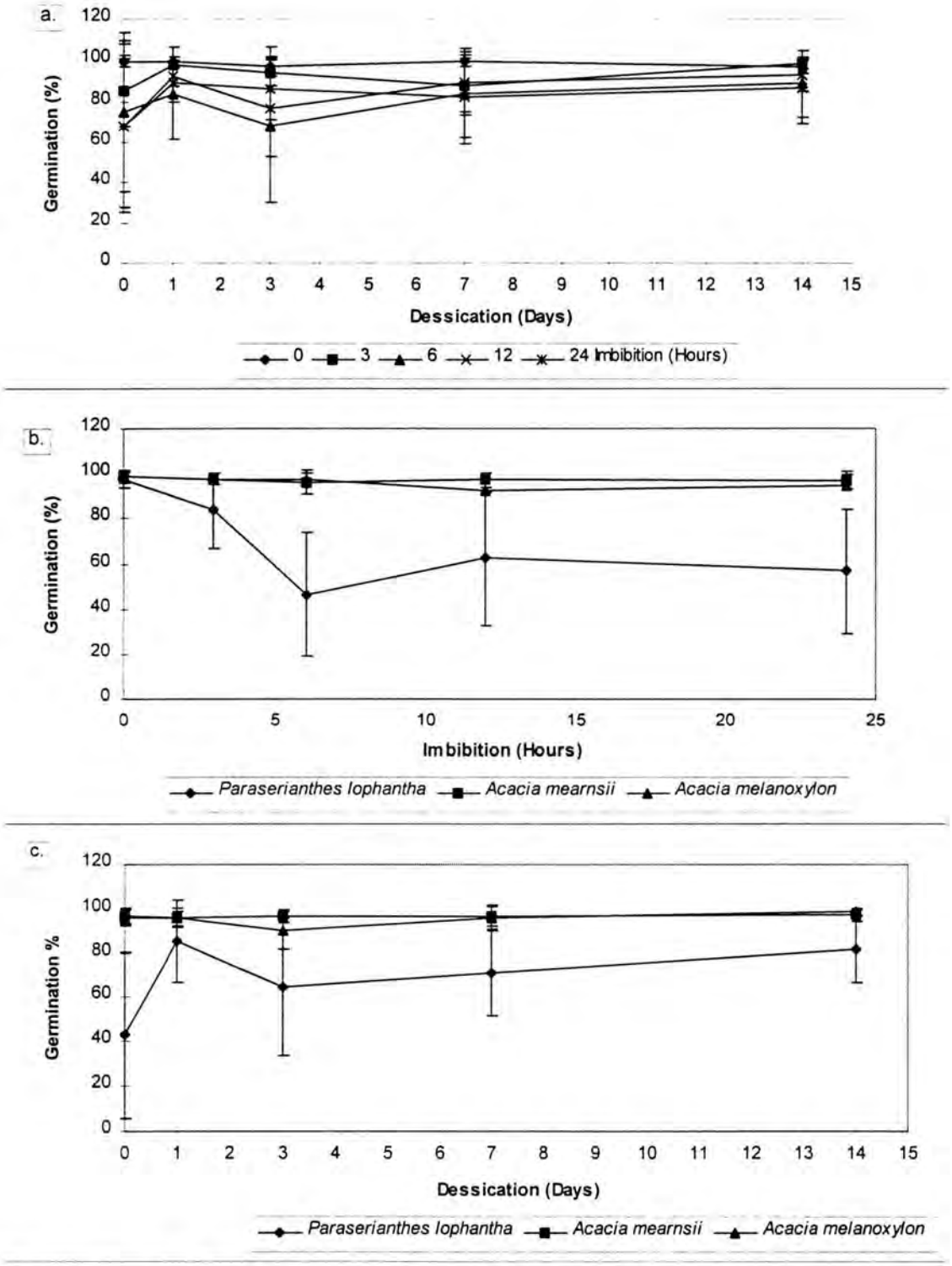

Figure 8 a. The effect of different desiccation and imbibition periods on the germination of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. **b.** The effect of different imbibition periods on the germination of *A. mearnsii*, *A. melanoxylon* and *P. lophantha*. **c.** The effect of different desiccation periods on the germination of *A. mearnsii*, *A. melanoxylon* and *P. lophantha*. (Vertical bars indicate standard deviation).

Regarding radicle growth, a significant (P=0.0001) interaction occurs between species, desiccation period and imbibition period. The results confirmed the results of germination percentage, in that *P. lophantha* radicle length is negatively influenced by all imbibition periods (Figure 9a). *A. melanoxylon*, and especially *A. mearnsii*, are not influenced by imbibition periods (Figure 9b,c). The increase in radicle length of *P. lophantha* and *A. melanoxylon* with the 0 hour imbibition/3days desiccation and 0 hour imbibition/1 day desiccation treatments respectively, makes no sense. Because no pre-treatment is involved, the desiccation treatment is merely a case of the seed being stored one or three more days before being incubated to germinate. Treatments are planned so that germination of all the treatments starts on the same day under the same conditions. The enhanced radicle growth of *A. mearnsii* with the 3 hours imbibition/1 day desiccation treatment, might therefore be a valid response to the treatment.

Effect of various solutes with varying osmotic potentials

There is no significant (P=0.1762) interaction between species, chemical compound and water potential. Significant interactions, however, occur between species and chemical compound (P=0.0221), species and water potential (P=0.0001) and chemical compound and water potential (P=0.0001). The interaction between chemical compound and species, although statistically significant, is difficult to observe visually (Figure 10a).

All species obtain the highest germination percentage in mannitol solutions, although *A. melanoxylon* appears to be the most sensitive to mannitol. NaCl treatments result in higher germination percentages than PEG, but the difference is marginal in the case of *P. lophantha*. *P. lophantha* appears to be more sensitive to low water potentials of -0.5 and -1 MPa, but at -1.5 MPa, *A. melanoxylon* all but ceases to germinate (Figure 10b). *A. mearnsii* appears to be most resistant to any of the osmotic stresses induced. Figure 10c confirms the indications of Figure 10a, that mannitol has the least inhibitory effect on the germination percentage of the three chemical compounds, except at the -0.2 MPa level. PEG and NaCl have about the same effect on germination percentage, except at the lowest water potential, where NaCl give marginally better germination percentages than PEG.

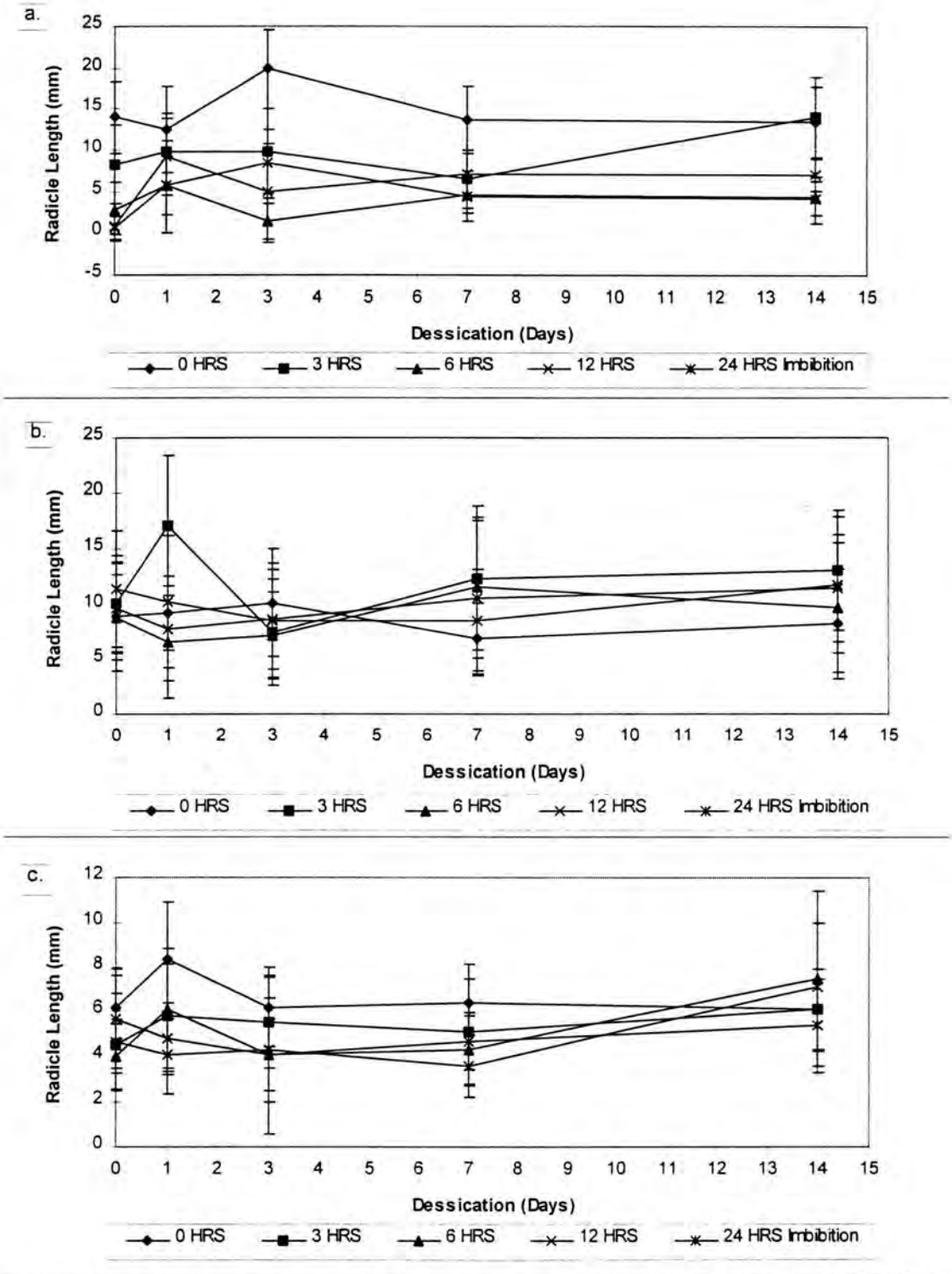

Figure 9 The effect of varying imbibition and desiccation periods on the radicle growth of **a**. *Paraserianthes lophantha*, **b**. *Acacia mearnsii* and **c**. *A. melanoxylon.* (Vertical bars indicate standard deviation).

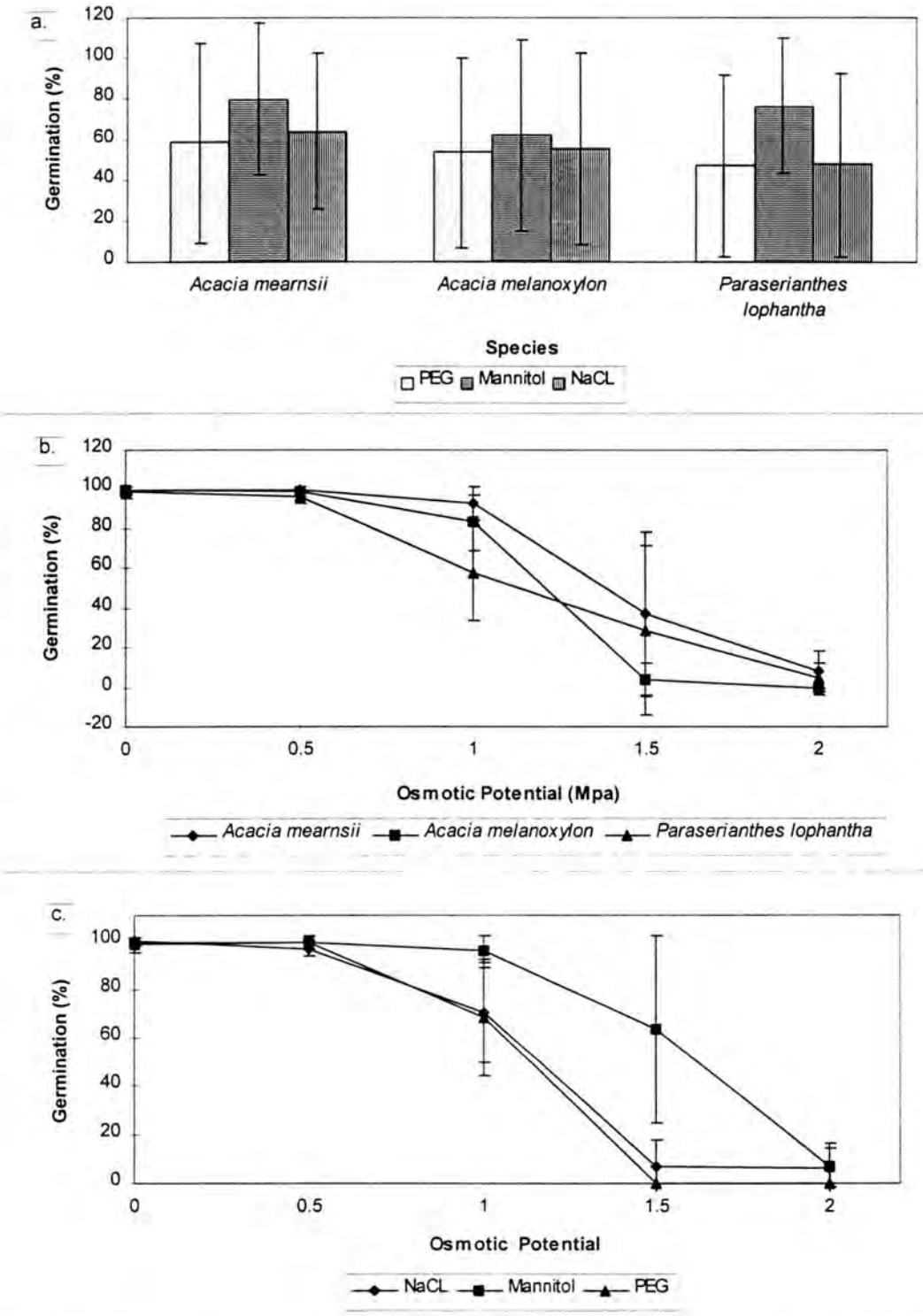

Figure 10 **a.** The effect of different osmoticums on the germination of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. **b.** The effect of different water potentials on the germination of *A. mearnsii*, *A. melanoxylon* and *P. lophantha*. **c.** The effect of different water potentials obtained with different chemical compounds on the germination of *A. mearnsii*, *A. melanoxylon* and *P. lophantha*. (Vertical bars indicate standard deviation).

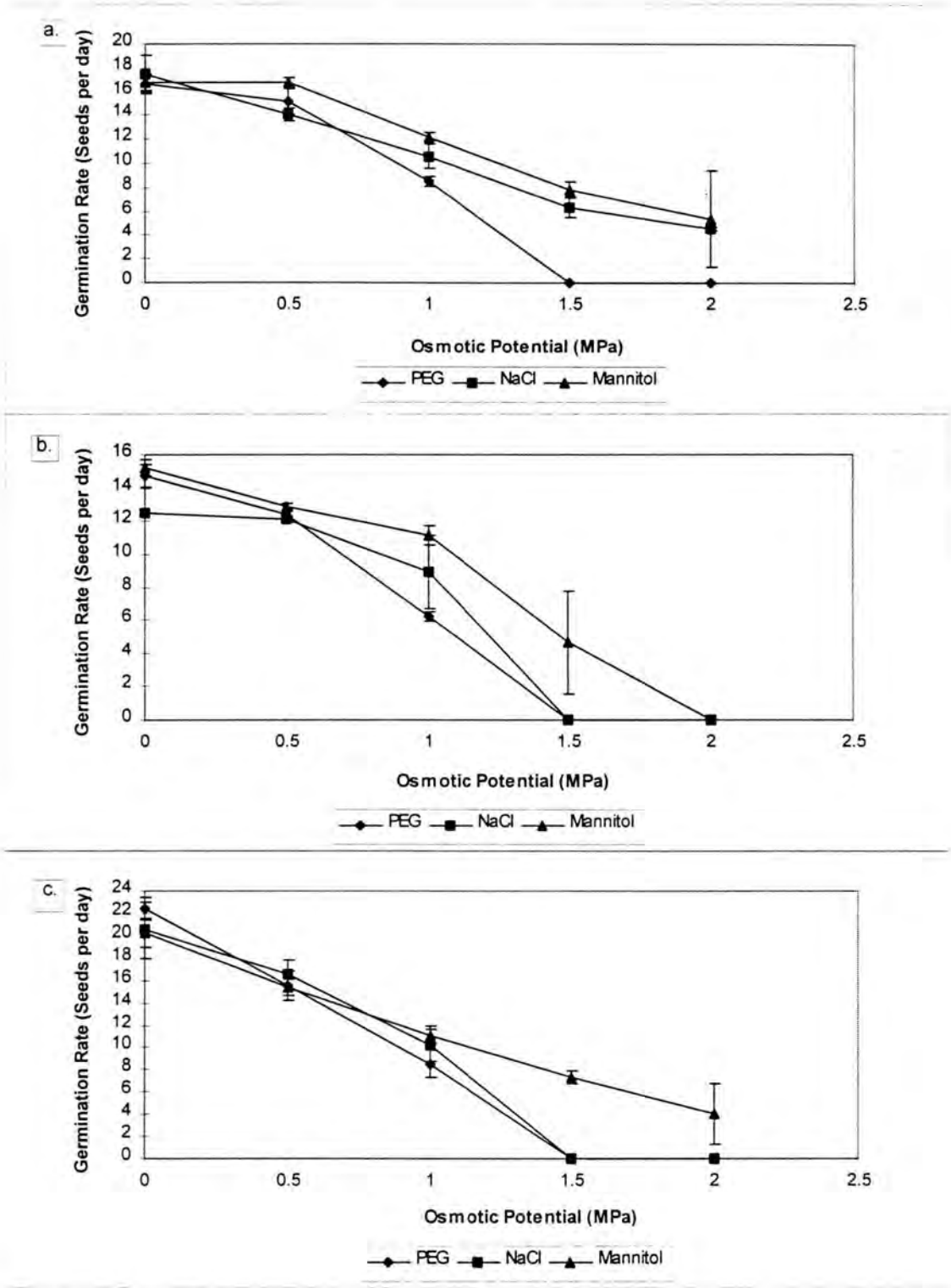

Figure 11 The effect of varying water potentials induced by different osmoticums on the germination rate of **a**. *Acacia mearnsii*, **b**. *A. melanoxylon* and **c**. *Paraserianthes lophantha*. (Vertical bars indicate standard deviation).

Regarding germination rate, a significant interaction (P=0.0001) between species, chemical compound and water potential occurs. Effects of treatments on germination rate (Figure 11a,b,c) largely corresponds to the effects of treatments on germination percentage (Figure 10a,b,c). PEG appears to have the most deleterious effect on the germination rate in all the species, whereas mannitol has the least serious effect. NaCl is less detrimental to *A. mearnsii* than to the other two species at water potentials of -1.5 and -2.0 MPa.

3.4 Discussion

Results of the first three experiments shows that light has no effect on the germination of the seeds of any of the species. This is not unexpected, as several workers have reported that legume seeds are not light-dependent for germination once the seed testa becomes water permeable (Preece 1971; Johnstone *et al.* 1979; Milton & Hall 1981; Graaff & Van Staden 1983b).

However, Taylorson (1982) states that it is safe to assume that every seed contains some phytochrome or the mechanism for making it, but it cannot be asserted that phytochrome always functions during the germination process. Except for seeds having barriers to water uptake and larger seeded species (e.g. more than several mm in diameter), germination control by phytochrome control is generally the rule in wild species (Taylorson 1982). The seeds of all three species investigated comply with these two conditions present in seeds that are not controlled by phytochrome. Kelley *et al.* (1992), however, refer to *Dichrostachys cinerea*, a legume with relatively large seeds, that does show improved germination after exposure to light. Bell and Van Staden (1993) confirmed this phenomenon and also found that there appears to be a loss in sensitivity to germination temperatures over time for *D. cinerea*. It is an example of the existence of a more complicated dormancy mechanism than just seed coat impermeability. Therefore, although these studies confirm the belief that germination of legume seeds is light independent, it is possible that phytochrome could be triggered by some or other treatment, such as high temperatures (Taylorson 1982), to control germination.

Bell (1994) on the other hand, while investigating the seed germination requirements of fire-cue requiring understorey species in western Australia, found that germination was similar in light and dark conditions at 13°C for two *Acacia* species, but at 18°C and above germination achieved under 12 h light was significantly lower than that achieved in complete darkness. Rokich and Bell (1995) found contrasting reactions to light in different *Acacia* understorey species, but their results also confirmed that phytochrome can play a role in the germination of legumes.

A. melanoxylon germination appears to be more negatively influenced by extreme temperatures than the other two species. This might be due to the fact that *A. melanoxylon* occurs in mesic forests in its country of origin, where the forest has an ameliorating effect on extreme temperatures. The seed of *A. melanoxylon* is therefore not adapted to germinate under such extreme temperatures. It is however interesting to note the drastic reduction in germination when the temperatures increase from 35 to 40°C. Germination of *A. melanoxylon* is almost normal at 35°C, in contrast to germination of *A. mearnsii* which is negatively influenced from a temperature of 30°C and higher. However, *A. mearnsii* germinates better than *A. melanoxylon* at 40°C. The rapid response of *A. melanoxylon* to elevated germination temperatures could be ascribed to a loss of viability, but it could also be a matter of phase transition of phytochrome at a temperature of 35 - 40°C (Taylorson 1982). Jansen and Ison (1994b) found that temperatures in the range of 30-40°C inhibited seed germination in most lines of *Trifolium resupinatum* and *T. balansae*, but that the same seed germinated readily when transferred to temperatures of 15°C. This seed was considered to possess an enforced high-temperature dormancy. *Trifolium* seeds exposed to temperatures of 45 - 50°C for longer than 8 hours, however, died. In some legume species, whether they have a permeable testa or not, germination is inhibited even upon hydration, and dormancy is maintained by hormonal control (Van Staden *et al.* 1989). It is therefore possible that *A. melanoxylon* has a light requirement at temperatures higher than 35°C. In another study *A. melanoxylon* was found to have a significantly lower germination percentage after pre-treatments in water at 40°C (Chapter 4). It was ascribed to an inexplicable loss of viability, whereas it could well have been a phase transition of phytochrome that inhibited germination. In that case, germination experiments were also performed under dark conditions. In a follow-up

investigation where *A. melanoxylon* seed were subjected to temperatures of 40°C and germinated under light and dark conditions, however, the phenomenon did not occur again (Chapter 4).

P. lophantha appears to be able to germinate well at temperatures from 5-40°C. It is somewhat surprising that *P. lophantha* is more resistant to higher germination temperatures than *A. mearnsii*, because it has been shown that *A. mearnsii* can endure higher temperatures for longer periods when seeds were given high temperature pre-treatments (Chapter 4). Seed under dry conditions can withstand much higher temperatures than seed with a high moisture content (Mayer & Poljakoff-Mayber 1982). This was illustrated by the work of Onwueme and Adegoraye (1975). It is therefore possible that although *A. mearnsii* is more heat resistant in the dry condition, it is more sensitive to elevated temperatures than *P. lophantha* in the imbibed condition.

The range of temperatures at which *P. lophantha* can germinate, is almost similar to those found by Eastin (1984) for *Sesbania drummondii*. Eastin concluded that *S. drummondii* will therefore be able to germinate right through the year. *P. lophantha* will probably behave similarly and should be able to germinate anywhere in South Africa. *A. melanoxylon* on the other hand, had a very low germination percentage at 5°C. The lack of germination below 10°C is characteristic of cold-sensitive plants. The effect of low temperatures on germination is probably caused by changes to the membrane permeability and activation energy of membrane enzymes (Hill & Luck 1991).

The germination percentage of plants in chronologically set duration experiments can however be misleading. A slow germination rate can mask the effects of temperature on percentage germination e.g. in the light/temperature experiment, germination of *A. melanoxylon* appears to have been totally inhibited by a temperature of 10°C (Figure 4), whereas in the next experiment, a germination percentage of over 80% was recorded at 10°C (Figure 5a). The reason for this discrepancy is the duration of the experiments. In the first case, the experiment was terminated after 8 days whereas in the second case, the first counts were made after 14 days. The duration of an experiment can therefore influence the results. It is thus probably better when

investigating the effect of temperature on germination, to use the concept of degree-days rather than a chronologically set duration experiment (Romo & Eddleman 1995). According to these authors, degree-day experiments give a better indication of the ecological implications of different germinating temperatures, where germination processes slow down and germination percentages in chronologically set duration experiments are an underestimation of the germination potential of the seed.

Some seeds require fluctuating temperatures to germinate (Popay & Roberts 1970; Thompson *et al.* 1977; Brecke & Duke 1980; Mayer & Poljakoff-Mayber 1982). An extreme case is the need for water impermeable seeds to be subjected to high temperatures for germination (Fenner 1985). In the case of the three species under investigation, the need for a short, high temperature pre-treatment has been shown (Chapter 4). In this study, however, the effect of fluctuating temperatures during germination is investigated. The three species germinate well under constant temperatures and do not need fluctuating temperatures for germination to take place. A few interesting phenomena are however noticed. The negative effect that temperature fluctuation amplitudes of 15°C have on *A. mearnsii* and *P. lophantha* is inexplicable. The seeds suffered from severe fungal attack at the higher temperatures, in spite of the fungicide mixture in the germinating solution. It was observed earlier that more fungal growth took place under light conditions than under dark conditions. Therefore, more fungal growth could have taken place where seeds were subjected to 12 hours of light in conjunction with the higher day temperatures than under constant temperatures in the dark. It can, however, be argued that *A. melanoxylon* was not influenced in the same way under similar conditions. This is an aspect that needs clarification.

The imbibition patterns of the seeds can be explained as follows: exposure of air-dry seeds to free water with a water potential of zero sets up a very high potential gradient and imbibition results. There is an initial period of rapid uptake which can almost be considered an uncontrolled influx of water, then follows a slower, linear phase of water uptake lasting several hours after which imbibition slacks off and finally comes to a halt (Simon 1984). The next mass-increase takes place when germination starts. The *Acacia* species reach the end of imbibition much faster than

P. lophantha, while the lag phase, before germination starts, is much shorter in *P. lophantha*. The actual process of germination therefore starts earlier in *P. lophantha*, irrespective of the fact that the initial imbibition period takes longer to complete. The relative rate of water uptake is fastest in *A. mearnsii*, followed by *A. melanoxylon* and *P. lophantha*. One can assume that the osmotic potential of the seeds decreases in the same order and therefore that resistance to high osmotic potential solutions would also decrease in the same order. Nevertheless, it appears as if *P. lophantha* might germinate faster than the other two species.

The germination of *A. mearnsii* and *A. melanoxylon* is not influenced by any of the imbibition or desiccation pre-treatments. Williams (1980) found that an increase in the number of hydration/dehydration cycles decreased the germination percentage of *Sesbania exaltata* seed. Jansen and Ison (1994a) found that a desiccation-tolerant period of imbibition for perennial *Trifolium* species existed. This was also determined for *Lotus corniculatus* (McKersie & Stinson 1980) and *Glycine max* (Senaratna & McKersie 1983). Berrie and Drennan (1971), Preece (1971), Vincent and Cavers (1978) and Horowitz and Taylorson (1984) generally found that short dehydration periods had no negative effects on the germination success of various plant species. *P. lophantha* seeds start germinating earlier than the seeds of the two *Acacia* species. It is possible that the desiccation-tolerant period of imbibition of *P. lophantha* is much shorter than those of the other two species. *P. lophantha* is influenced most by desiccation after imbibition periods of longer than three hours. If *P. lophantha* starts to germinate earlier than the other two species, as indications are, desiccation could have taken place after cell division and elongation started. The worst effect of desiccation is however after 6 hours of imbibition. One has therefore to assume that *P. lophantha* does not have a desiccation-tolerant imbibition phase.

It must be borne in mind that in this investigation, seeds were completely immersed under water in a glass beaker during the imbibition phase. Therefore the results are not directly comparable to those of other workers who used moist filter paper during the imbibition period. It is possible that *P. lophantha* becomes depleted of oxygen after three hours immersion in water, resulting in a lower germination percentage. On the other hand, *P. lophantha* might be the only species that can develop further under

water, and was therefore damaged by desiccation, whereas the two *Acacia* species stopped the germination process as soon as the rapid imbibition phase was over, and therefore did not develop past the desiccation-tolerant stage.

It appears as if the technique used to create an osmotic water stress, is successful. Emmerich and Hardegree (1991) warn that filter paper exclusion of polyethylene glycol and water vapour loss significantly influence results when germination tests are run in the usual way where a few millimetres of germination solution are added to two or three filter paper disks in a petri-dish. In this case enough solution is added to the petri-dish to ensure that there is an amount of free solution about 1-2 mm deep available in the petri-dish. The petri-dishes are enclosed in an airtight polyethylene bag to prevent evaporation. The free solution is however shallow enough not to cover the seeds completely, as that may lead to a lower total germination percentage due to oxygen deficiency (Hardegree & Emmerich 1994).

Osmotic stress reduces the total germination percentage of all three species, as was found for several other species (Hoveland & Buchanan 1973; Williams 1980; Fyfield & Gregory 1989). It is somewhat surprising that PEG has a greater influence on germination percentage than NaCl and mannitol, because Sharma (1973) found that NaCl and mannitol could have a possible toxic effect and that PEG is more suited to determine the effect of drought on seed germination. Hampson and Simpson (1990a) however found that out of a range of salt osmoticums, NaCl treatments were least inhibitory for wheat germination, permitting a greater germination percentage than PEG treatments at all but the highest water potential. Temperature stress intensified the effects of osmotic stress and *vice versa*. The observed salt-temperature interactions in wheat could have been due to changes in membrane leakage (Hampson & Simpson 1990b). Corchete and Guerra (1986) found no difference in the effect of PEG and NaCl stress treatments on the germination, solute content and enzyme activities in lentil seeds. The relative effect that PEG and NaCl have on seed germination is therefore probably influenced by the species under investigation. The mild effect of NaCl in relation to the other two chemical compounds is still a little surprising, because even a halophyte like *Atriplex semibaccata* displays reduced germination at elevated NaCl concentrations (De Villiers *et al.* 1994).

No definite conclusions can be made about the effect of water potential tension on the different species. It appears as if *A. mearnsii* is most resistant to water potential stress, *P. lophantha* is least resistant at the lower stress levels while *A. melanoxylon* is least resistant at the highest stress levels. Response of germinating seed to osmotically induced water stress can however not be used unconditionally to determine drought resistance of plants, because factors such as particle size and soil water conductivity also plays a role (Sharma 1973). A better indication of drought resistance of plants will probably be obtained using a soil-membrane-polyethylene glycol system like the one used by Kaufmann (1969), because it determines the matric, and not the solute effects on germination.

The apparent sensitivity of *P. lophantha* to lower water stress levels relative to the other species, could be a result of physiological differences between species, or it could be the result of the bigger seed size of *P. lophantha*. Hadas (1976) mentioned that large seeds that absorb more water from a limited volume of osmoticum, can cause a further reduction in water potential. Hucl (1993) found that bean genotypes with the narrowest seeds tended to germinate faster and to a greater degree at suboptimal temperatures and under moisture stress. In this study, however, the relatively large volume of osmoticum added to the petri dish should have been enough to counteract such problems.

Failure of seeds to germinate under moisture stress could also be an induced light dependency as was found for *Citrullus lanatus* (Botha *et al.* 1984). They concluded that the inhibitory effect of water stress on seed germination of *C. lanatus* was due to the prevention of the interaction of P_{fr} with the hypothetical component x, which is necessary for the conversion of phytochrome. As seeds in this experiment were germinated in the dark, such an inhibitory action is possible at low water potentials.

Fungal attack is another factor that can reduce the overall germination percentage of seed. At higher moisture stress levels, prolonged germination can make the seeds more vulnerable to fungal attack, as was reported by Hunter and Erickson (1952). In this experiment, massive rotting of seeds due to fungal attack occurred at the higher moisture stress levels. This was probably due to a combination of prolonged germination, and the absence of fungicide in the germination solution. The result of

these fungal attacks was that seeds could not be transferred back to pure water to determine whether the solutions just inhibited germination, or were in fact toxic to the seeds. Whether the seed mortality that took place was due to fungal attack or to toxicity of the osmoticums, is therefore unclear.

The sensitivity of *P. lophantha* to desiccation can be considered when planning control measures. Currently the control measures against most of these invasive species includes fire, which is preferably applied in late summer to autumn, when fire is less detrimental to indigenous flora (Seydack & Bekker 1993). However, if *P. lophantha* seeds could be stimulated to germinate in late spring or early summer in the mediterranean area of the south-western Cape of South Africa, there is a good chance that the seeds will undergo one or more desiccation cycles which may cause considerable mortality among the seed population. This, however, will only be possible in areas where low cover of indigenous vegetation occurs.

In conclusion, it appears as if *P. lophantha* is tolerant of the widest range of germinating temperatures whilst *A. melanoxylon* is the least tolerant. Both these species appear to be more sensitive to drought stress than *A. mearnsii*. The hypothesis stating that *A. melanoxylon* will have a narrower amplitude regarding germination conditions is therefore accepted. From these germination characteristics it appears as if *A. mearnsii* has the potential for the widest distribution over various climatic areas. However, germination requirements do not always indicate the requirements of the seedlings or adult plants correctly. For instance, from the germination temperature data it can be concluded that *A. mearnsii* will germinate and therefore grow in areas with lower minimum temperatures than *A. melanoxylon*, which should not be cold-tolerant. The fact is that *A. melanoxylon* has been observed to grow in frost-prone areas in the south-western Cape (Elgin) and in the eastern parts of the Free State, a province of South Africa where *A. mearnsii* does not grow. In Australia, *A. melanoxylon* grows in some areas where frosts are frequent and snow can be a regular feature (Farrell & Ashton 1978). However, taking this into consideration, it is still presumed that *A. mearnsii* will be a better competitor under most climatic circumstances in South Africa and is therefore the most serious invasive plant to which urgent attention should be given, both in terms of control, but also in terms of

finding ways to utilise the plants, which in itself is a way of control. The other two species, especially *P. lophantha*, however, appear to be able to establish and grow in a wide range of climates and caution must be exercised when these plants occur near pristine indigenous vegetation.

Chapter 4

Relationship between mode of dispersal and germination responses to heat and acid treatments of three co-occurring invasive legumes

4.1 Introduction

The success of invasive plants can be largely attributed to their reproductive success in the absence of the natural enemies in their country of origin (Hughes & Styles 1989). To control the abundance of these plants, the reproductive capacity will have to be limited (Milton & Hall 1981). Invasive species belonging to the Fabaceae, generally produce seeds in which water-impermeability is imposed by the seed coat (Rolston 1978; Werker 1980/81). In the absence of seed-feeding insects, these-long lived seeds can build up large soil-stored seed banks (Dean *et al.* 1986). Under certain conditions, especially after fire, these seeds can give rise to massive seedling populations (Pieterse & Cairns 1986). The seed of most of the invasive *Acacia* species in South Africa are adapted to germinate after fire (Milton & Hall 1981). The seed of some species, like *Acacia cyclops*, however, are not stimulated to germinate after fire (Holmes 1989b). In order to plan successful control strategies, it is important to gain a better understanding of the dormancy, germination characteristics and reaction to dormancy-breaking treatments of these species.

Acacia mearnsii De Wild., *A. melanoxylon* R. Br. and *Paraserianthes lophantha* (Willd.) Benth. are important invaders of the fynbos biome (Macdonald & Jarman 1984). These three species have more or less the same habitat requirements and can therefore occur in and compete for the same growing space. Control options for these species will probably not vary substantially. The reaction of the seeds of these plants to different dormancy-breaking treatments can be important, because an integral part of a control programme will be to control the soil stored seed bank, either by killing the seeds or stimulating them to germinate. From an ecological point of view it will be interesting to

compare the reaction of the species to dormancy-breaking treatments, as *A. melanoxylon* is a bird-dispersed species (Davidson & Morton 1984; O' Dowd & Gill 1986) and *A. mearnsii* is an ant-dispersed species (O' Dowd & Gill 1986). *P. lophantha* is considered to be an ant-dispersed species because the diaspores contain elaiosomes (Pemberton & Irving 1990), but is probably not primarily ant-dispersed (Chapter 2).

The objectives of this study are to gain a better knowledge of the germination requirements of the species which can assist in the development of effective control management programmes, and to determine whether evolutionary adaptation to different dispersal agents might have caused differences in the seed coat characteristics, and therefore in the dormancy characteristics of the species. The following hypothesis is tested: There is no relationship between dispersal adaptation and dormancy breaking mechanisms.

4.2 Materials and methods

In December 1989, ripe seed were harvested from *A. mearnsii*, *A. melanoxylon* and *P. lophantha* trees in the vicinity of Stellenbosch (33° 56' S; 18° 52' E). These were stored for about 6 months in glass jars under dark conditions at room temperature before being used in germination experiments to test the effect of wet and dry heat. Seeds were again harvested from the same localities in December 1990 for further tests with the same methods and for acid scarification experiments.

Hot water treatments

Four replicates of 25 seeds per treatment of each of the three species were subjected to the following treatments; seeds were immersed for 0, 1, 2.5, 5, 10, 30 and 60 minutes into water at constant temperatures of 40, 60, 80 and 100°C. Seeds were enclosed in a fibreglass mesh bag and lowered into the water for the appropriate time. Sufficient water was used to prevent temperature fluctuations associated with the immersion of the seeds. The seeds were then transferred directly into 90 mm plastic petri-dishes containing two filter paper discs and 6 ml of a benomyl\captab solution (0.022% and 0.055% m/v a.i. respectively) (Clemens *et al.* 1977). The petri-dishes were then enclosed in poly-ethylene

bags to prevent moisture loss. The bags containing the petri-dishes were then incubated in a growth chamber in the dark at a constant temperature of 20°C. Petri-dishes were inspected every third day and germinated seed (*i.e.* seeds with radicle protruding more than 1 mm), were recorded and removed from the petri-dishes. Rotten seeds were also recorded and removed. After 102 days, when no further germination took place, seed that had not imbibed or germinated, were chipped at the distal end with a sharp blade and incubated for a further 14 days. The percentage of seeds that germinated was calculated. The number of seeds that germinated after being chipped, were considered to be the number of viable seeds because Pieterse (1986) established that 100% germination can be obtained from *A. longifolia* seed following mechanical scarification by chipping the distal end. Seeds that did not germinate after being chipped, were not considered to be viable.

Dry heat treatments

Four replicates of 25 seeds per treatment of each of the three species were subjected to one of the following treatments; seeds in glass petri-dishes were put into a preheated oven for 0, 1, 2.5, 5, 10, 30 and 60 minutes at temperatures of 40, 60, 80, 100, 150 and 200°C. The seeds were transferred directly after treatments into 90 mm plastic petri-dishes and germinated in the manner described above. Seeds that failed to imbibe or germinate after 115 days were chipped at the distal end and incubated for a further 14 days. Germination percentage were calculated as described above.

Wet and dry heat treatments in sand

Three replicates of 25 seeds per treatment of each of the three species were subjected to the following treatments; glass petri-dishes that were filled with pure potting soil and were either oven-dried beforehand or wetted to field capacity. Seeds were placed so that each seed had 5 mm of sand above and below it. The petri dishes with the sand and seeds were then inserted into a pre-heated oven at 100°C for 0, 5, 10, 30 and 60 minutes. After the petri dishes with sand had cooled sufficiently, the seeds were separated from the sand and were germinated as described above. After 75 days seeds which had not imbibed or

germinated were chipped at the distal end and incubated for a further 14 days. Germination percentage and rate of germination were calculated as previously described.

Response of *A. melanoxylon* to pre-treatments at 40°C

Results from the previous experiments indicated that dry heat treatments at 40°C either kill *A. melanoxylon* seeds or possibly induce a secondary dormancy condition (Figure 3a). To investigate the possibility of the seed being transferred into a dormant state after dry heat treatments, the following experiment was carried out. Six replications of 25 seeds were treated as follows: 1) Seed was subjected to dry heat by incubating them in an oven at 40°C for 30 minutes; 2) Seed was subjected to wet heat by immersing them into water at a temperature of 40°C for 30 minutes. After the treatments, two replications were subjected to the tetrazolium test for viability as described in Chapter 1 and four replications were incubated to germinate under light and dark conditions at a constant temperature of 25°C (to speed up germination) in the manner described above. The light source consisted of florescent tubes and one 100 w. incandescent light bulb that resulted in a light intensity of 25.5 μ mol s^{-1} m^{-2}. For the light treatment the petri-dishes were inserted into clear polyethylene bags and for the dark treatment the petri-dishes were wrapped in heavy duty aluminium foil. Both treatments were incubated in the same growth cabinet. Seven days after incubation, the petri-dishes were opened and the number of seeds that germinated was recorded. The non-germinated seeds were subjected to the tetrazolium test to determine viability. Experimental design was a 2x2 factorial with factors heat and light.

Acid treatments

Six replications of 25 seeds per treatment of each of the species were subjected to one of the following treatments; seeds were immersed into concentrated sulphuric acid for 0, 10, 30, 60, 120, 240, 360 and 480 minutes. The seeds were then rinsed for 2 minutes under running tap water and were then transferred to petri-dishes and germinated as described. Seeds that were not imbibed or germinated after 30 days, were chipped at the distal end

and incubated for a further 14 days. Germination percentage and rate of germination were calculated as before.

Statistical analysis

Data was analysed by means of the LSMEANS command of PROC GLM (SAS Institute Inc. 1985). Germination data were logit-transformed before analysis.

4.3 Results

Hot water treatments

Significant interactions in germination percentage occur between water temperature and exposure period (P=0.0078) and between species and water temperature (P=0.0001). The same interactions occur when comparing percentage viability (P=0.0248 and P=0.0001 respectively).

Seeds immersed in water at 40° and 60°C show a small increase in germination percentage with a longer exposure period, while seeds immersed in water at 80°C show a slight decrease and at 100°C a significant decrease in germination percentage with longer exposure periods (Figure 1a). Increasing exposure periods at the lower water temperatures cause increasing loss of water impermeability in seeds, with a subsequent increase in germination. At the higher temperatures however, increasing exposure periods also affect the viability of the seeds (Figure 1b), and the percentage germination decreases in spite of the seed testa becoming permeable to water (indicated by the imbibition of the seeds).

The interaction between water temperature and species expose interesting trends. Water temperatures of 60 and 80°C appears to be the optimum temperatures to induce germination in *A. melanoxylon* and *P. lophantha* (Figure 1c), although the germination percentages are less in water temperatures of 80°C in the case of *A. melanoxylon* seeds (Figure 1d). *A. mearnsii*, on the other hand, requires temperatures of 80 and 100°C to effectively increase germination (Figure 1c) and viability is not decreased by water

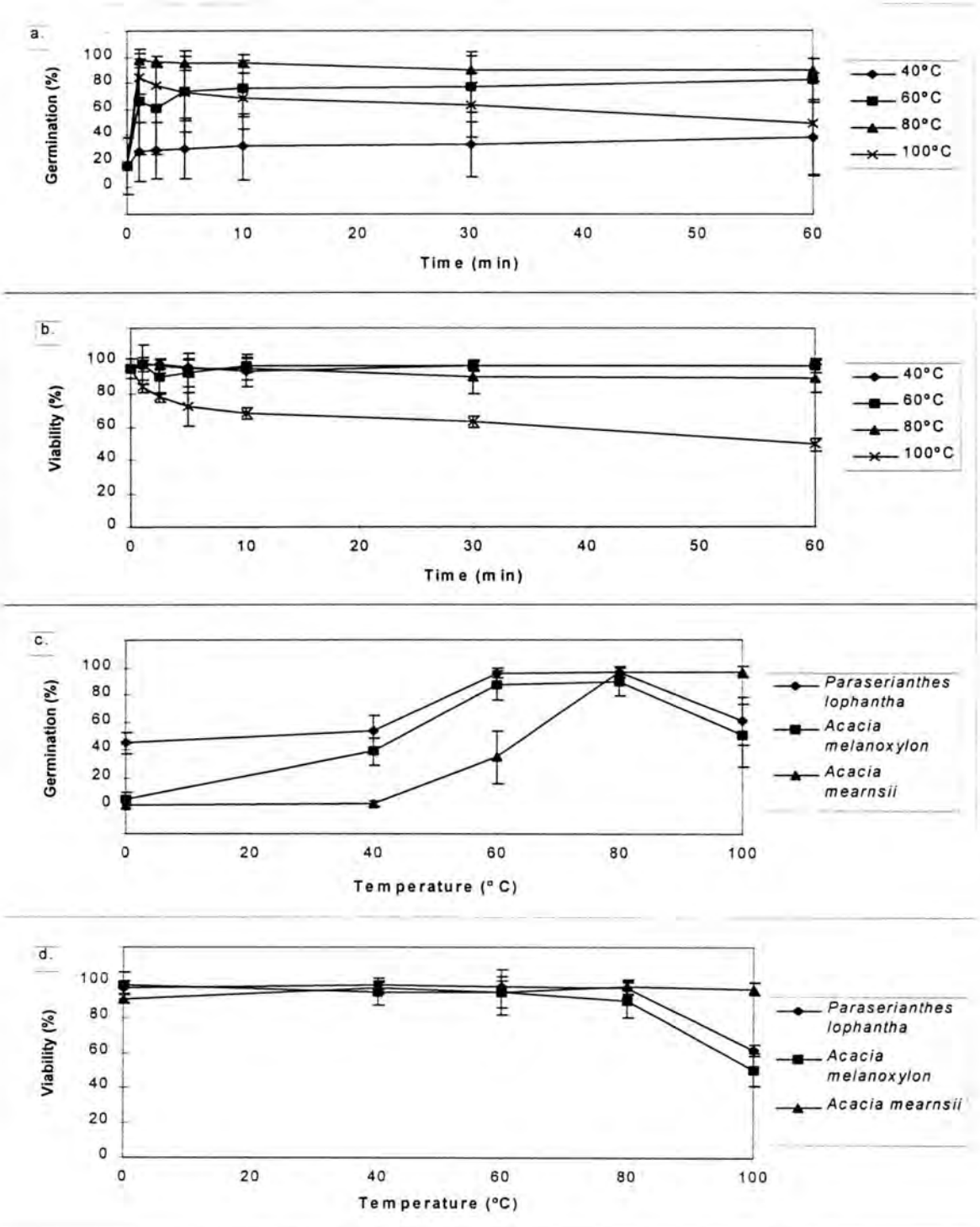

Figure 1. The effect of varying exposure periods in water of different temperatures on: **a**. the seed germination and **b**. the seed viability of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha*. **c**. The effect of water temperature on the seed germination and **d**. seed viability of *A. mearnsii, A. melanoxylon* and *P. lophantha*. (Vertical bars indicate standard deviation).

temperatures of 100°C as in the case of the other two species (Figure 1d). The control experiment shows that all species have a high percentage of viable seed and that the two *Acacia* species have a high percentage of water-impermeable seeds (Figure 1). In contrast, almost half of the seeds of *P. lophantha* are water-permeable.

Dry heat treatments

Significant interactions occur between species, oven temperature and exposure period (P=0.0001) for both germination percentage and percentage viability. Results from the dry heat treatments are more erratic than those from the hot water treatments, but the general trends correspond. Again *P. lophantha* germinate better at the lower temperature treatments than the *Acacia* species but again the water-permeability of the untreated seeds is significantly higher than those of the *Acacia* species (Figure 2a,b). Germination of *A. mearnsii* is stimulated only after longer exposure periods at 80°C and is not influenced by the lower oven temperatures (Figure 2a,b,c). The lower oven temperatures generally do not influence the viability of the seeds although the viability of *A. melanoxylon* is inexplicably low in this particular experiment at the 40 and 60°C treatments. It is highly unlikely that the treatments could have destroyed the seeds because viability is not influenced dramatically by the 80°C treatment (Figure 3a,b,c). All the seeds used were from the same batch and therefore the viability before treatment should have been high. The seeds that did not germinate did not rot, as other seeds did when their viability was destroyed, but stayed imbibed and ungerminated for the duration of the experiment after being chipped. The seeds reacted as though secondary dormancy could have been induced. This was investigated in a following section. The longer exposure periods at 80°C and 100°C decrease the viability of especially *A. melanoxylon* seeds, and to a lesser extent, those of *P. lophantha*.

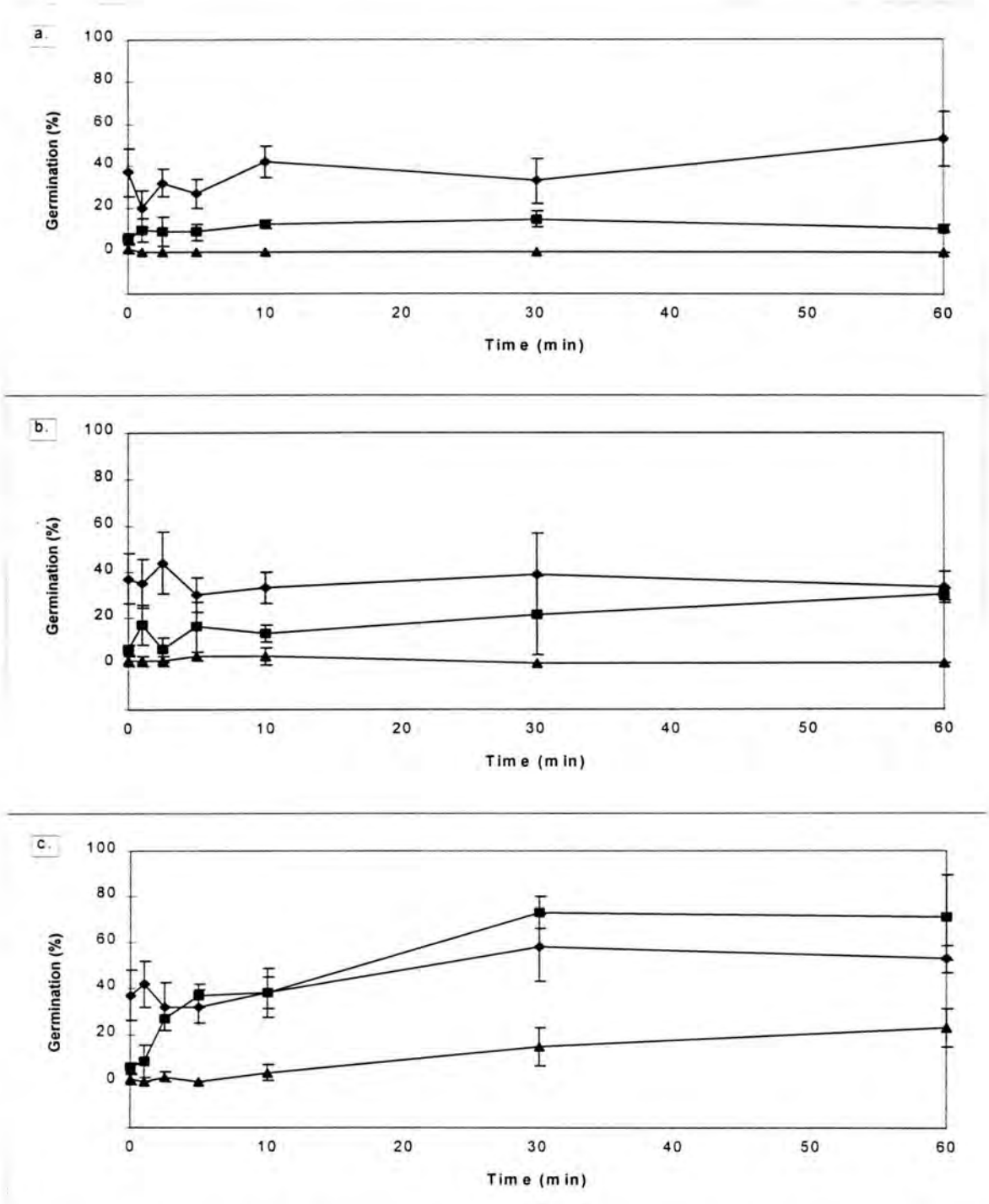

Figure 2. The effect of varying exposure periods to dry heat in an oven at different temperatures on the germination of seeds of *Acacia mearnsii* (Δ), *A. melanoxylon* (■) and *Paraserianthes lophantha* (◆). **a.** 40°C. **b.** 60°C. **c.** 80°C. (Vertical bars indicate standard deviation).

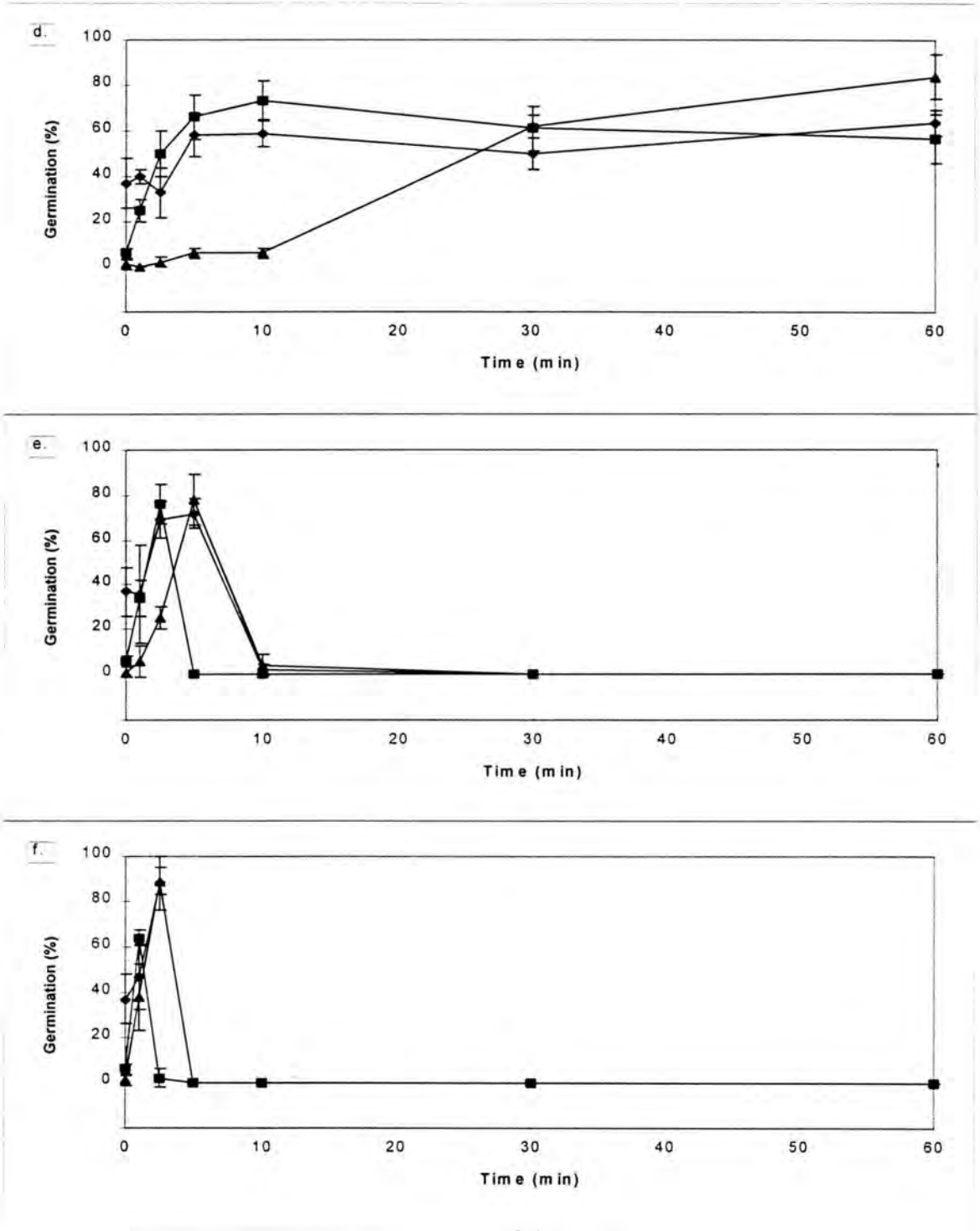

Figure 2 continued. The effect of varying exposure periods to dry heat in an oven at different temperatures on the germination of seeds of *Acacia mearnsii* (Δ), *A. melanoxylon* (■) and *Paraserianthes lophantha* (◆). **d.** 100°C. **e.** 150°C. **f.** 200°C. (Vertical bars indicate standard deviation).

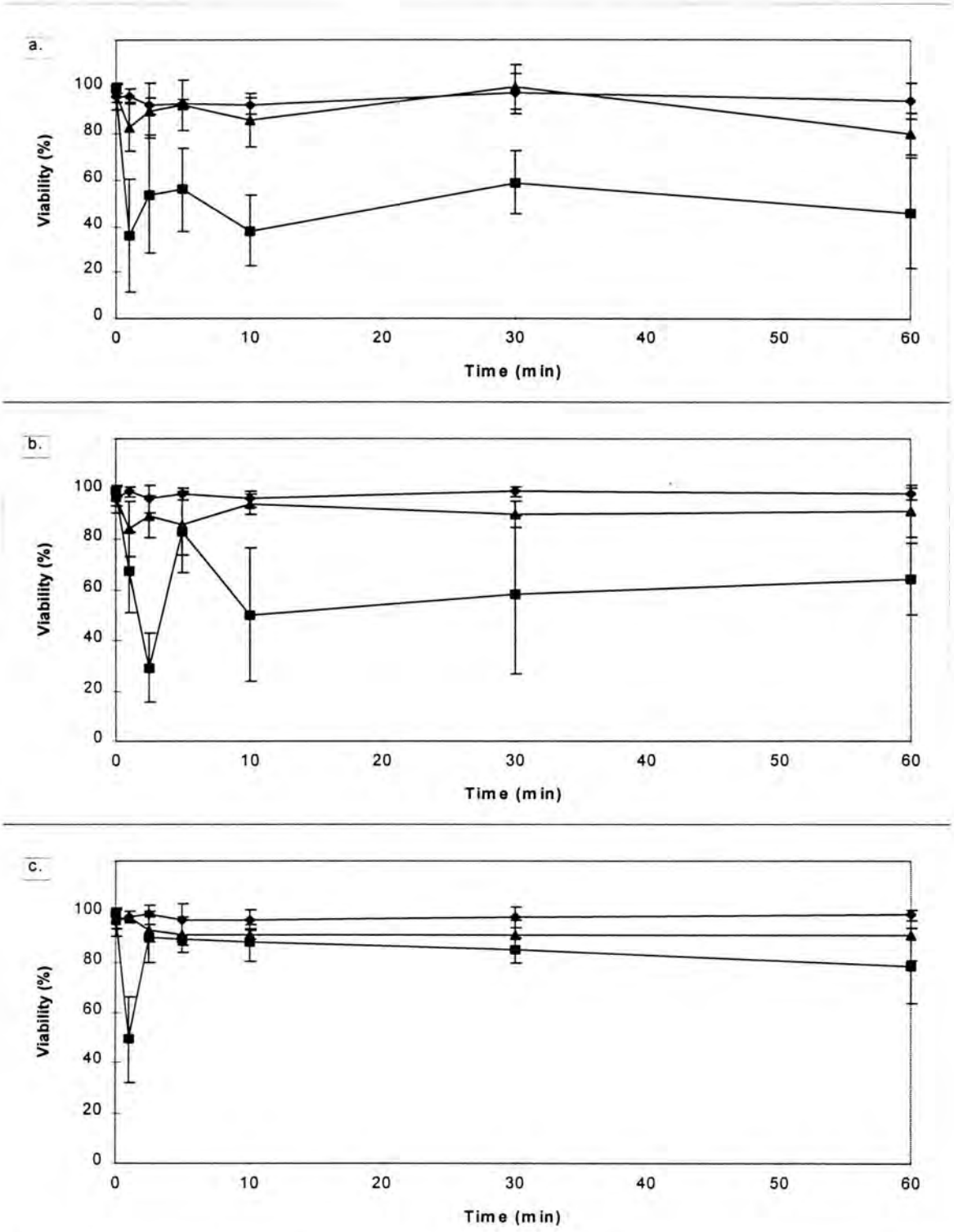

Figure 3. The effect of varying exposure periods to dry heat in an oven at different temperatures on the viability of seeds of *Acacia mearnsii* (Δ), *A. melanoxylon* (■)and *Paraserianthes lophantha* (◆). **a.** 40°C. **b.** 60°C. **c.** 80°C. (Vertical bars indicate standard deviation).

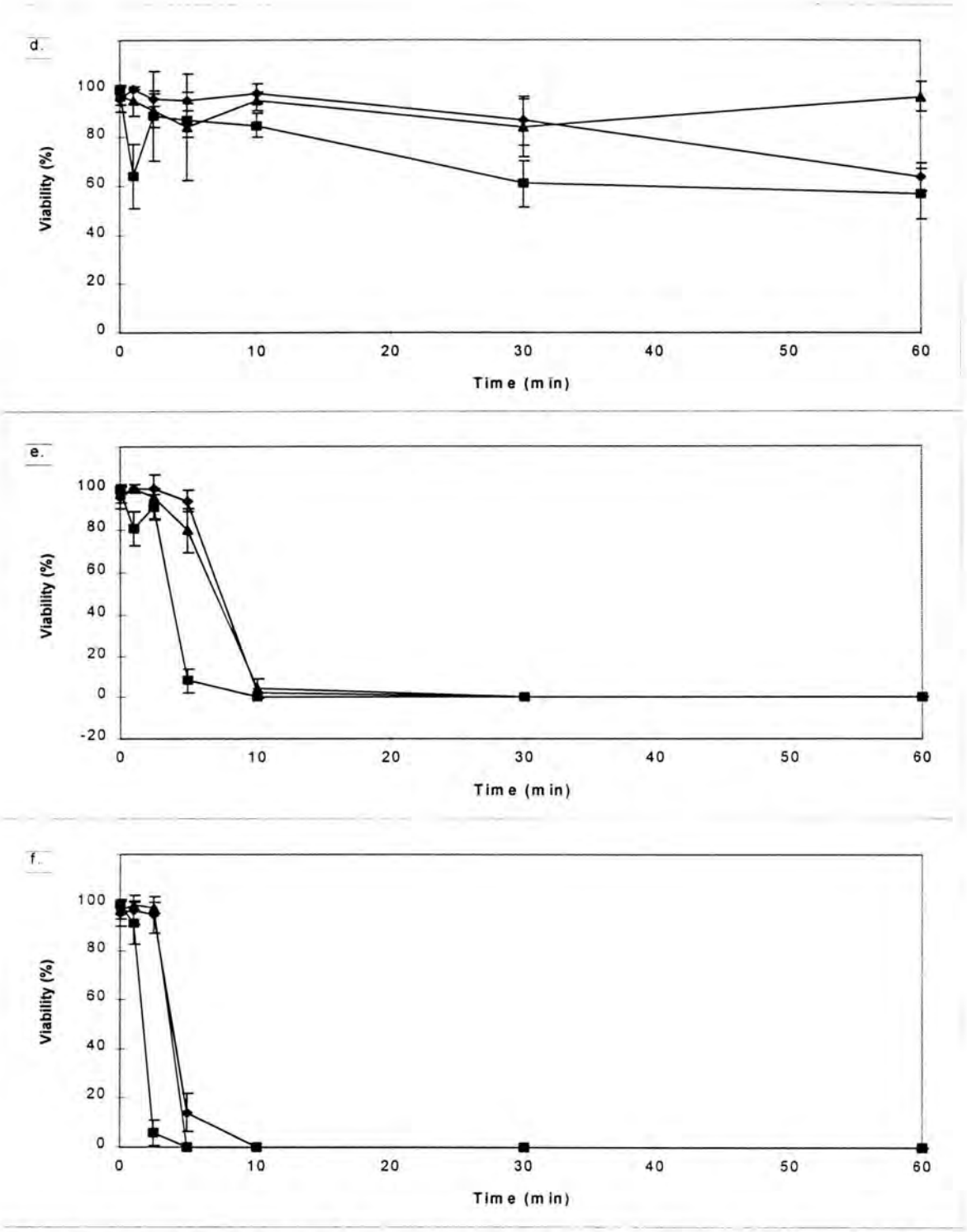

Figure 3 continued. The effect of varying exposure periods to dry heat in an oven at different temperatures on the viability of seeds of *Acacia mearnsii* (Δ), *A. melanoxylon* (■) and *Paraserianthes lophantha* (◆). **d.** 100°C. **e.** 150°C. **f.** 200°C. (Vertical bars indicate standard deviation).

The viability of *A. mearnsii* is not influenced by even 60 minutes at 100°C (Figure 3c,d). At the highest (150 and 200°C) temperatures, the 1, 2.5 and 5 minute and the 1 and 2.5 minute exposure periods respectively increase germination (Figure 2e,f), while the longer exposure periods destroy the viability of the seeds (Figure 3e,f).

Wet and dry heat treatments

Significant interactions occur between heat treatment (wet or dry) and exposure period (P=0.0001), heat treatment and species (P=0.0001) and between species and exposure time (P=0.0002) for germination percentage. Viability interactions only occur between heat treatment and exposure period (P=0.0001) and species and exposure period (P=0.0021).

Wet heat treatments are more effective in stimulating germination than dry heat treatments (Figure 4a). The failure of dry heat treatments to stimulate germination at longer exposure periods is not a failure to render seeds water-permeable, but is caused by the destruction of the viability of the seeds (Figure 4b). The relatively low germination response of *A. melanoxylon* to longer exposure periods (Figure 4c) is also a result of a loss of viability under these conditions (Figure 4d). *A. mearnsii* has a better germination percentage with dry heat than with wet heat (Figure 4e), in contrast with the other two species. Figure 4f shows that no significant interaction (P=0.5437) occurs between heat treatments and species in respect of viability. Dry heat consistently results in a lower viability percentage, which is especially marked in the case of *A. melanoxylon*.

Response of *A. melanoxylon* to pretreatments at 40°C

No interaction occurs between heat and light treatments (P=0.001). The seed in all treatment combinations give a high (>90%) germination percentage and no significant differences (P=0.001) between either wet and dry heat, or light and dark germination conditions can be detected. Seeds that do not germinate is damaged by fungal/bacterial attack. Tetrazolium staining shows that more than 95% of seeds are viable after the heat treatments.

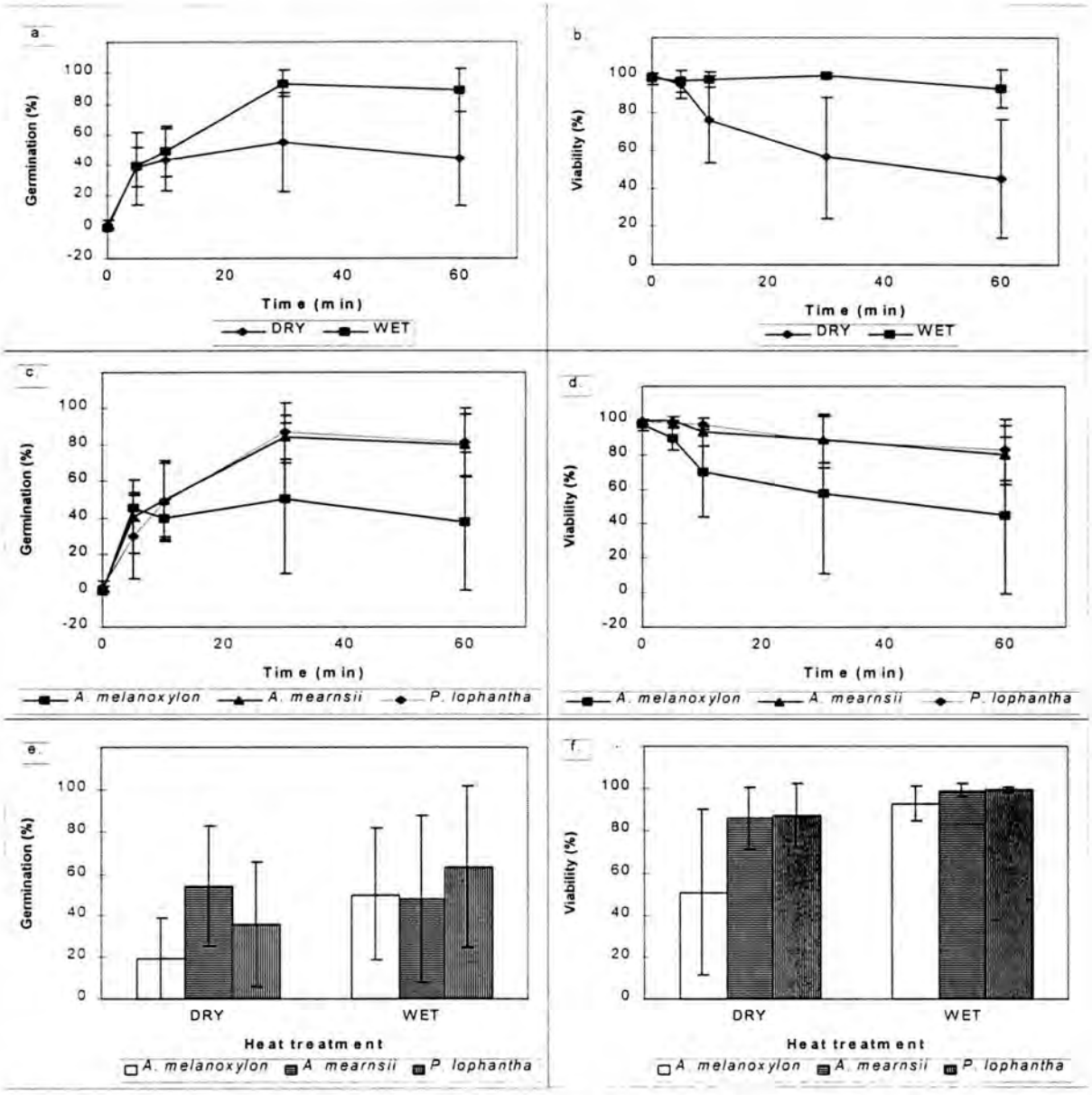

Figure 4. The effect of varying exposure periods in wet and dry sand at 100°C on the **a**. germination and **b**. viability of seeds of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha*. **c.** The effect of varying exposure periods in wet and dry sand at 100°C on the germination and **d**. viability of seeds of *A. mearnsii, A. melanoxylon* and *P.lophantha*.. **e.** The effect of wet and dry sand at 100 °C on the germination and **f**. viability of seeds of *A. mearnsii, A. melanoxylon* and *P. lophantha*. (Vertical bars indicate standard deviation).

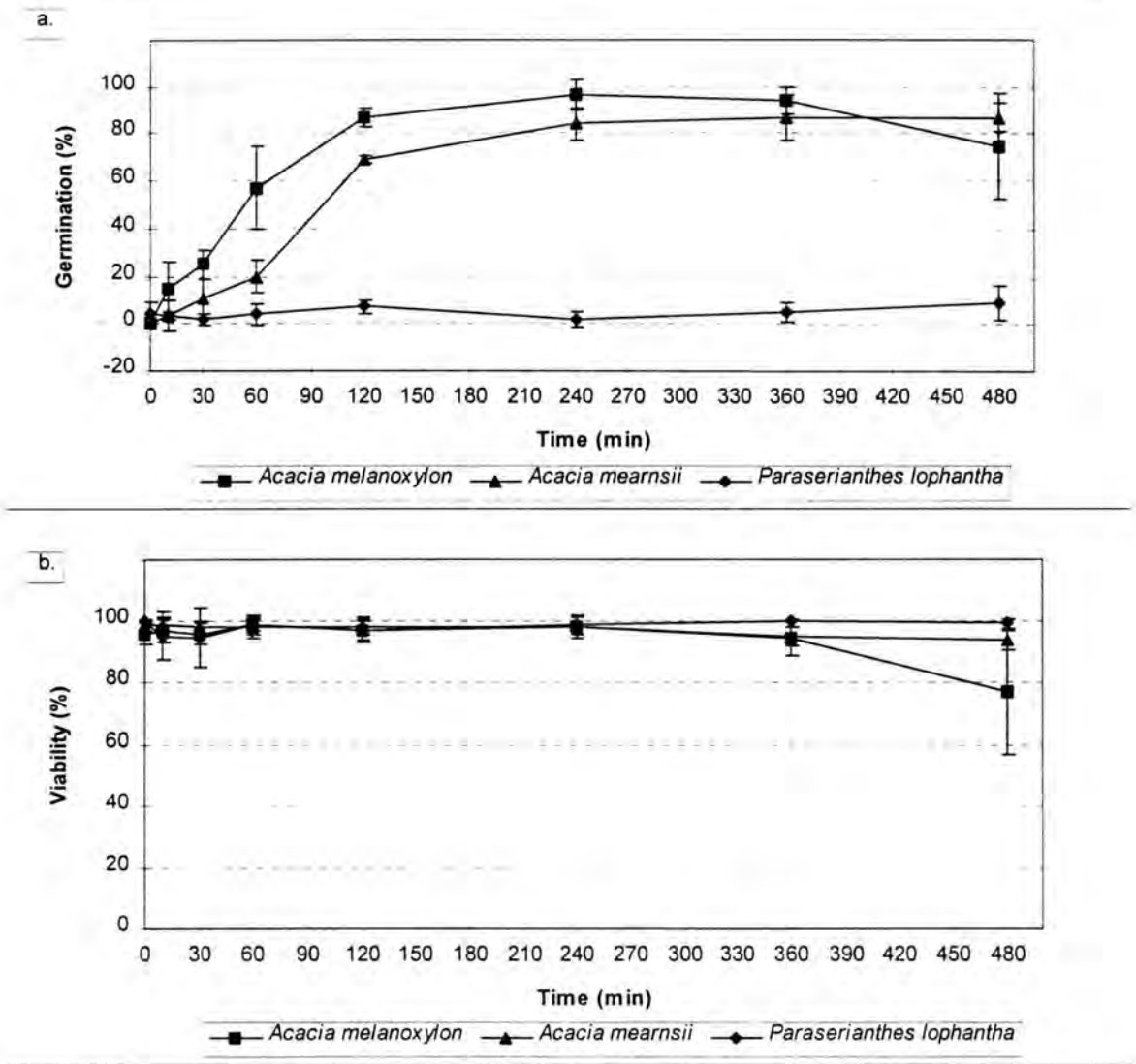

Figure 5. The effect of varying exposure times in concentrated sulphuric acid on the **a**. germination and **b**. viability of seed of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha*. (Vertical bars indicate standard deviation).

Acid treatments

A significant (P=0.0001) interaction occurs between species and exposure period to concentrated sulphuric acid. *P. lophantha* appears completely resistant to acid treatment and even after 8 hours of soaking, germination percentage and viability of the seeds remain unchanged (Figure 5a,b). *A. melanoxylon* appears to be the most sensitive to sulphuric acid treatment because relatively more germination takes place at shorter

immersion periods while viability is influenced more acutely at the longer immersion periods (Figure 5a,b).

4.4 Discussion

The results of the hot water treatments correspond to those found by other workers. Hot water treatments increased permeability of legume seeds like *Acacia kempeana* (Hanna 1984), *Albizia* (=*Paraserianthes*) *lophantha* (Dell 1980), *A. mearnsii* (Gupta & Thapliyal 1974; Hendry & Van Staden 1982) and *A. melanoxylon* (Gupta & Thapliyal 1974). Hot water treatments of 60°C cause germination in about 90% of *A. melanoxylon* and *P. lophantha* seeds while the corresponding percentage germination with dry heat at the same temperature is about 25% and 35% respectively. *A. mearnsii,* too, germinates at lower temperatures where hot water is applied than where dry heat is applied. Horowitz and Taylorson (1984) found the same trends in *Abutilon theophrasti*, a hard-seeded member of the Malvaceae. *A. mearnsii* appears to be the most resistant to both hot water and dry heat treatments. Several authors subjected seeds to dry heat treatments to investigate the effect that fire would have on the soil seed bank (Shea *et al.* 1979; Auld 1986a; Jeffery *et al.* 1988). Martin *et al.* (1975) however stated that application of moist heat is a better simulation of forest fires than dry heat. They also found that some *Cassia* species responded to moist heat but not to dry heat. Results from dry heat treatments applied in an oven as in the dry heat experiment and in the study of Jeffery *et al.* (1988), cannot be compared to hot water treatments as there is closer contact and better conduction of heat from water to the seed than from hot air to the seed. The wet and dry heat treatments in sand give better indications of how wet and dry heat would affect seed permeability and viability under natural conditions. Figure 4a shows that extended periods of wet heat exposure achieve better germination percentages than exposure to dry heat, but the failure of dry heat treated seed is due to a decrease in viability of the seed (Figure 4b). Heat treatments also damage *A. melanoxylon* after shorter periods than the other two species. It appears as if *A. mearnsii* seed is better adapted to heat than *A. melanoxylon*, with *P. lophantha* somewhere in between. The apparent loss of viability of *A. melanoxylon* after 40°C temperatures is inexplicable and might have been attributed to

a secondary dormancy enforced by the particular heat treatment. Although the dormancy of hardseeded species is quite simple, and most seeds germinate after the seed testa has been rendered permeable by some scarification action, there are exceptions, for example, Kelly *et al.* (1992) found that *Dicrostachys cinerea* seed, after the seed coat was rendered water-permeable, had a light requirement for germination to take place. However, the investigation of the effect of wet and dry heat at 40°C on *A. melanoxylon* seed, shows no indication that the seed is either killed or transferred into a secondary dormancy condition. An in-depth study of the reaction of *A. melanoxylon* seed to heat treatments may clarify the results depicted in Figure 3a.

On the other hand, *A. melanoxylon* responds better to acid treatments than *A. mearnsii* while *P. lophantha* does not respond at all. The poor response of *P. lophantha* to acid treatment can be attributed to the relatively thick seed testa (*ca.* 400µm) compared to the seed testas (*ca.* 200-250µm) of the two *Acacia* species (Chapter 2). Acid treatments had the following effect on the seed coat of *Sesbania punicea* (Manning & Van Staden 1987); 1) the cuticle was removed but no obvious damage to the epidermis was visible; 2) scattered localized damage, including deep cracks occurred; 3) the hilar region was penetrated and tracheid bar was damaged and 4) extensive damage to the lens occurred, and a median fissure formed through the epidermis. Sealing of the lens after scarification did inhibit germination, but not completely, indicating that in some seeds the lens was the area where the seed became permeable, but in other seeds the acid must have damaged other parts of the testa. Johnstone *et al.* (1979) found that the thickness of the testa of *S. exaltata* did not decrease measurably after 60 min acid scarification, but the germination percentage was 99%. According to them scarification could therefore be affecting the hilum. Kelly and Van Staden (1985) showed that acid scarification damaged the macrosclereid layer of *Aspalathus linearis*. Graaff and Van Staden (1983a) showed that seed of *S. punicea* could remain impermeable, in spite of a deeply etched epidermis, indicating that whatever is responsible for the seed coat impermeability, extends down to some depth in the macrosclereid layer. Graaff and Van Staden (1983b) attributed the longer duration of acid scarification necessary to make *S. punicea* seed permeable (90

min vs. 30 min for *S. bispinosa*) to the thinner seed coat of the latter (450µm and 120µm respectively). The testa thickness of *P. lophantha* is comparable to that of *S. punicea*, but even 8 hours of acid scarification did not improve permeability markedly. In contrast, *P. lophantha* reacted well to heat treatments. Heat treatments cause permeability of legume seed testas by damaging a weak area of the testa, the lens (Tran 1979; Dell 1980; Hanna 1984; Tran & Cavanagh 1984; Manning & Van Staden 1987; Van Staden *et al.* 1989). It, therefore, does not appear as if the thickness of the testa plays an important role in heat sensitivity of seed. The sensitivity of the lens to heat, irrespective of testa thickness, is the main factor involved in the permeability of legume seed to water after heat treatment.

The relatively higher sensitivity of *A. melanoxylon* to acid scarification could be related to the composition of the seed testa. The macrosclereid layer of *A. melanoxylon* constitutes only about 25% of the total testa thickness, whereas in *A. mearnsii* and *P. lophantha* the macrosclereid layer constitutes 35% and 40% of the total testa thickness respectively (Chapter 2). If acid scarification affects the macrosclereid layer as found by, amongst others, Manning and Van Staden (1987), a shorter exposure to acid will be sufficient to render *A. melanoxylon* seeds permeable. The loss of viability at longer exposure periods is possibly due to the fact that most of the seed testa is disintegrated by the acid, and uncontrolled and uneven imbibition causes cellular rupture of the seeds, with subsequent loss of viability (Duke & Kakefuda 1981). It is also possible that the embryo is damaged by the acid scarification. The chemical and physical bonding of the macrosclereid cells will probably play a role in the resistance of seed testas to acid scarification. If testa thickness or the thickness of the macrosclereid layer alone influenced the duration of acid scarification needed to render a testa permeable, *P. lophantha*, having a testa roughly twice the thickness of the other two species, should become permeable after 8 hours of acid scarification. *P. lophantha* has a lignified area outside of the light line which does not occur in the palisade layers of the other two species (Chapter 2) and which probably plays a role in the resistance of *P. lophantha* to acid treatments.

In Australia *P. lophantha* occurs in "karri-forest" along roadsides, on seashore, sandy and lateritic soil (Nielsen *et al.* 1983b). It is a mesophytic understorey tree and has a frequency governed by the periodicity of fire (Dell 1980). *A. mearnsii* occurs in open forests and cleared areas, especially on drier shallow soils, while *A. melanoxylon* occurs on a variety of sites, but is best developed on deep soils, especially in tall forests and "jungle" pockets in mountain valleys (Costermans 1981). It appears therefore that *A. mearnsii* and *P. lophantha* occur in more open, xeric conditions and would therefore be more exposed to wildfires than *A. melanoxylon*. All three species have seeds that are fire-adapted, judging from the reaction to heat treatments, but *A. melanoxylon* seed is more susceptible to heat than the other two species. The seed of *A. melanoxylon* will probably be dispersed by forest bird species, and it is therefore likely that they will be deposited in forests under trees that serve as bird perches. The sensitivity to acid scarification indicates an adaptation to bird-dispersal although O' Dowd and Gill (1986) maintain that no co-evolved traits unique to ants or birds are involved in the protection of seeds during dispersal. A study of the heat and acid sensitivity of a wider range of bird- and ant-dispersed species might confirm or dismiss the hypothesis that there is a correlation between mode of dispersal and sensitivity to acid or heat scarification. Milton and Hall (1981) found that *A. cyclops*, a bird-dispersed species, required two hot water treatments to germinate. The insensitivity of *A. cyclops* to heat treatments was confirmed by the results of heat treatments by Jeffery *et al.* (1988) and by the effect of burning on *A. cyclops* seed banks (Holmes *et al.* 1987; Holmes 1989b). Jones (1963) found that *A. cyclops* did react to hot water treatments, albeit slower than the reaction of *A. saligna* (= *A. cyanophylla*). Glyphis *et al.* (1981) suggested that dormancy of *A. cyclops* is more effectively broken by acid scarification.

If such a simple explanation of the behaviour of *A. melanoxylon* is true, the behaviour of *A. mearnsii* and *P. lophantha* is harder to explain. The larger seed of *P. lophantha* with large cotyledons and thicker testa should be more resistant to heat damage than smaller seeds such as those of *A. mearnsii* (Sabiiti & Wein 1987). The results of this study contradict that assumption. Whether *P. lophantha* is a myrmecochore is an

important question. The low investment of *P. lophantha* into dispersal structures casts some doubt on its status as a myrmecochore (Chapter 2). If it is not a myrmecochore, as is suggested by this study, then the large majority of *P. lophantha* seeds should occur in the top 50 mm of soil, as is the case with *Acacia suaveolens* seeds that are not incorporated into ant's nests (Auld 1986a). *P. lophantha* should then be sensitive to temperatures of 40-80°C, such as occur in the top layer of soil during bush fires (Floyd 1966; Bradstock & Auld 1995). The average soil temperature at a depth of 10 mm caused by a fire in fynbos vegetation was 57.3°C with the highest temperature being 71°C (De Lange & Boucher 1993). *A. mearnsii*, a definite myrmecochore, is also adapted to fires. If it is presumed that most of the seeds will be stored in an ant's nest, it is curious that it only germinates after heat treatments of 80°C and more, because such temperatures will rarely occur below 50 mm depths. Auld (1986a) found that more than 40% of *A. suaveolens* seeds stored in ant's nests were buried deeper than 100 mm. Except in the case of extremely high intensity fires, temperatures will not rise to such levels at those depths (Floyd 1966). Monk *et al.* (1981) found that most *A. pulchella* seedlings established from 10-30 mm depths while Cunningham & Cremer (1965) found that 90% of *Acacia* seedlings emerged from depths of more than 12.5 mm. According to heat requirements for germination, as well as resistance to heat damage, *A. mearnsii* seeds are actually better adapted to shallow burial than to deep burial in an ant's nest.

Berg (1981) reasoned that dispersal and subsequent storage of seeds by ants permits the survival of the seeds through fire and furthermore may place seeds in a "safe site" for establishment after germination because of greater water and nutrient availability provided by ant activity. Auld (1986a) found that only 40% of *A. suaveolens* seed were buried, 60% of those buried were buried under rock shelves where emergence of germinated seedlings was impossible and that about 40% were buried deeper than 100 mm. Although *A. suaveolens* seed can germinate and emerge from 100 mm depths (Auld 1986b), any seeds buried deeper than 60 mm can be considered too deep for successful germination, because heat cannot reach that deeply, even from a hot fire (Auld 1986a). The mean depth of seedlings that established after a fire, was 24.4 mm with a range of 10

mm to 60 mm. Auld (1986a) concludes that adaptation for ant dispersal is then more of a disadvantage than an advantage.

The seed characteristics and their response to heat and acid scarification, in the context of their ecological adaptations, makes sense in the case of *A. melanoxylon* and *P. lophantha*, but not really in the case of *A. mearnsii*, except if it is accepted that *A. mearnsii* evolved mainly in reaction to the 60% of seeds that possibly escapes burial by ants. From a weed control point of view in South Africa, the ant-dispersal adaptation is beneficial to the survival of the species. In this case the control measures would mostly include fire (Pieterse 1987) to kill/stimulate the seeds to germinate in the soil stored seed bank. Depending on how deep the seeds are buried, they will escape stimulation by the fire and will survive till the dormancy is broken by natural means. Seed banks of *A. melanoxylon* and *P. lophantha* will therefore be easier to deplete by fire than those of *A. mearnsii*. Although excellent arguments against the use of fire in alien plant control has been put forward (Breytenbach 1989), fire must be considered under certain conditions. If a dense natural vegetation cover with low risk of wildfires can be guaranteed for an extensive period, fire can be omitted from an integrated control programme. However, in the south-western Cape where wildfires are the norm rather than the exception, the risk is always there that managers will end up with a mass of invasive seedlings the eradication of which has not been budgeted for in terms of money, labour and time. This scenario is even more feasible where parent plants have been eradicated a few years previously, and the threat of the seed in the soil seed bank has been forgotten about.

The intensity and duration of a fire, and therefore of the soil temperatures attained, will determine which species will re-vegetate a burnt area because their seeds react differently to different fire intensities and duration (Floyd 1976). In the case of *A. melanoxylon* and *P. lophantha*, a hot fire, which is detrimental to the soil and indigenous geophytes and sprouting shrubs (Breytenbach 1989), might be used because it might mean an almost complete eradication of the seed bank if it is assumed that the seeds only occur in the top layer of soil. In the case of *A. mearnsii*, cooler fires will probably be better, because all the seeds cannot be destroyed by the burn, and a cool fire is less detrimental to the

environment. However, this poses the threat that a later, hot fire might stimulate the remaining seeds in the deeper layers, which had escaped the effects of an initial cool fire, to germinate. These assumptions should be tested under field conditions to determine the most effective and economical control programmes.

In conclusion, there appears to be a relationship between mode of dispersal and most effective dormancy-breaking mechanism and the hypothesis is therefore refuted. This conclusion must however be tested using a bigger selection of species.

Chapter 5

Seed size as determinant of seedling establishment and early growth from different sowing depths

5.1 Introduction

Competition between co-occurring plant species can be severe in the seedling stage. The establishment rate and subsequent growth rate are crucial factors determining the outcome of inter specific competition (Harper 1977). In most cases seedlings that establish earlier have a competitive advantage over seedlings that establish later (Fenner 1985). Establishment of seedlings is obviously dependent on the availability of seed, but also on the frequency of so called "safe sites". Recruitment of seedling populations from seed banks is controlled by a sieve, which consists of an environmental lattice of safe and unsafe sites (Harper 1977).

There are numerous factors which affect the safety of a site for the establishment of a seedling e.g. availability of moisture, light, optimal temperatures, *etc.*; depth of seed burial is another one of these (Fenner 1985). Although the seed of a large number of species are inhibited from germinating at deeper depths by a light requirement for germination (Radosevich & Holt 1984), some seeds, like those of *Acacia mearnsii* De Wild., *Acacia melanoxylon* R. Br. and *Paraserianthes lophantha* (Willd.) Benth., which are investigated in this study, normally do not have a light requirement and can germinate at deeper burial depths. Considering the fact that these three species produce long lived, soil stored seed banks that can be stimulated to germinate by fire (Boucher 1978; Moll 1978; Taylor 1978), ability to germinate at and establish seedlings from deeper depths, can be a factor in inter-specific competition between these species.

In this study, the effect of different sowing depths as well as inter-specific interference on the establishment of seedlings of the above species were investigated. Observations were also made on the early growth of the seedlings after establishment, but a proper competition study could not be made due to the varying number of seedlings established. In this study, two hypotheses are tested: i) *P. lophantha*, having bigger seeds, will establish faster and from deeper sowing depths than *A. mearnsii* and

A. melanoxylon; ii) *A. mearnsii* and *A. melanoxylon*, having seeds of similar size, will have similar abilities to establish from various sowing depths.

5.2 Materials and methods

Seeds of *A. mearnsii*, *A. melanoxylon* and *P. lophantha* were scarified at the distal end with a sharp blade before sowing. For convenience these names are abbreviated to R, X and P below.

The treatment design was a 6x7 factorial; 48 seeds were sown in 7 litre nursery bags at one of six depths (10, 30, 60, 90, 120 and 150 mm) in one of seven possible mixtures amongst the species (P, X, R, PX, PR, XR, PXR). In the case of the monocultures (P, X, R) 48 seeds of a single species were sown. In the case of the bi-blends (PX, PR, XR) 24 seeds of each of the two species were mixed and sown in a bag, while in the case of the tri-blend (PXR) 16 seeds from each species made up the mixture.

Two replications of the 6X7 treatment combinations were arranged as a randomized block design in a glasshouse with a night/day temperature range of ca. 15/30°C. Two response variables were measured for each bag; the number of seedlings which established (of 48 possible) were determined at weekly intervals and the dry mass of the plants per bag two months after planting were determined by harvesting whole plants and drying them for two days at 80 °C before determining the dry mass.

Statistical analysis

Analysis of seedling establishment is done by making use of the **X** matrix used in the analysis of a continuous variable using a generalized linear model (McCullagh & Nelder 1983). For the analysis of dry matter, a model that is explained in Table 2 and 3 of Appendix 1, is fitted. An ANOVA analysis corresponding to the model is used to determine significant differences between treatment means. A detailed account of the statistical methods used are given in Appendix 1.

5.3 Results

Seedling establishment

Table 1 shows the numbers of seedlings that established after sowing of seeds of species at different depths and in different species mixtures. There is no significant difference between the seedling establishment of species in tri-blends in comparison to the seedling establishment of the species in monocultures.

Table 1. The number of seedlings established from 48 seeds planted at different depths in different combinations.

	DEPTH (mm)											
MIXTURE*	10		30		60		90		120		150	
P	30	35	21	29	32	24	29	30	21	28	0	5
X	43	42	20	34	2	2	0	0	0	0	0	0
R	43	45	42	44	30	30	11	6	1	4	0	1
PX:P	18	20	17	19	12	10	10	11	4	14	1	0
PX:X	24	22	19	22	12	7	0	0	0	0	0	0
PR:P	11	16	14	15	14	14	14	13	8	11	0	1
PR:R	24	21	23	22	22	18	16	15	1	0	0	0
XR:X	19	20	24	19	10	10	0	2	0	0	0	0
XR:R	22	24	22	24	16	23	7	12	0	3	0	0
PXR:P	10	7	11	11	9	11	9	9	4	4	0	0
PXR:X	15	16	15	14	9	9	1	1	0	0	0	0
PXR:R	13	16	16	16	14	13	6	6	4	0	0	0

*P=*Paraserianthes lophantha*
X=*Acacia melanoxylon*
R=A. *mearnsii*

Significant differences occur between the performance of *A. melanoxylon* and *A. mearnsii* in bi-blends and the same species in monocultures at the 30 mm and 60 mm depths (Table 2). *A. melanoxylon* establishes more seedlings in a mixture with *A. mearnsii* than in a monoculture at 30 mm and 60 mm depths while *A. mearnsii* produces slightly but significantly more seedlings in the mixture (Tables 1 and 3).

Table 2. The significance levels of terms of a model fitted sequentially to plot-total data on the number of seedlings establishing themselves in various mixtures of three species planted at one of six depths. Non-convergent models are marked "nc".

	df	10	30	60	90	120	150
Blocks**	1	0.2203	0.0230	0.3954	0.8675	0.0264	0.0237
PvR	1	0.0001	0.0001	0.0924	0.0001	0.0001	0.0057
PR	1	0.0076	0.6187	0.0001	0.0001	0.0011	0.8959
XvP&R	1	0.0916	0.0118	0.0001	0.0001	0.0001	0.0283
PX	1	0.9381	0.0001	0.0340	0.9537	0.0437	nc
XR	1	0.4691	0.0003	0.0001	nc	nc	nc
PXR	1	0.3376	0.6906	0.1183	nc	nc	nc
Residual	6	0.9598	0.1827	0.3220	0.0008*	0.0901*	0.5376*

* The residual deviance of the last convergent model. The residual degrees of freedom change accordingly. These are 6,6,6,8,8 and 9 respectively.

**P = *Paraserianthes lophantha*

X = *Acacia melanoxylon*

R = *Acacia mearnsii*

Table 3. The significance levels of terms of a model fitted sequentially to data per species on the number of seedlings establishing themselves in various mixtures of three species planted at one of six depths. Non-convergent models are marked "nc".

	df	10	30	60	90	120	150
Blocks**	1	0.2244	0.0025	0.3146	0.9105	0.0214	0.0018
PvR	1	0.0001	0.0001	0.0078	0.0001	0.0001	0.0063
PR:P	1	0.0001	0.3132	0.0595	0.0018	0.0097	0.6140
PR:R	1	0.6360	0.0259	0.0001	0.0001	0.5547	nc
XvP&R	1	0.1452	0.0019	0.0001	0.0001	0.0001	nc
PX:P	1	0.5169	0.4829	0.2322	0.1530	0.0042	nc
PX:X	1	0.0128	0.0115	0.0368	0.0406	nc	nc
XR:X	1	0.4016	0.0002	0.0016	0.5701	nc	nc
XR:R	1	0.1042	0.0024	0.0001	0.0007	nc	cn
PXR:P	1	0.0127	0.9716	0.0832	0.0131	nc	nc
PXR:X	1	0.1357	0.2658	0.0541	nc	nc	nc
PXR:R	1	0.7099	nc	0.0606	nc	nc	nc
Residual	11	0.0441	0.0474*	0.2004	0.7392*	0.0746*	0.9493*

* The residual deviance of the last convergent model. The residual degrees of freedom change accordingly. These are 11, 12, 11, 13, 17 and 20 respectively.

** P = *Paraserianthes lophantha*

X = *Acacia melanoxylon*

R = *Acacia mearnsii*

In the case of the *A. melanoxylon/P. lophantha* bi-blends, significant differences in the number of seedlings that established in the bi-blends and monocultures occur at the 30, 60 and 120 mm depths (Table 2). *A. melanoxylon* produces significantly higher numbers of seedlings in the bi-blends at the 30 and 60 mm depths than in the monocultures while *P. lophantha* produces significantly less seedlings in the mixture at 120 mm than in the monoculture at that depth (Tables 1 and 3). In the *P. lophantha/A. mearnsii* biblend, significant differences in the number of seedlings produced between the bi-blends and the monocultures occur at 10, 60, 90 and 120 mm depths (Table 2). At 10, 90 and 120 mm *P. lophantha* establishes significantly less seedlings in bi-blends than in monocultures while at 60 and 90 mm depths, *A. mearnsii* has a higher number of seedlings establishing in the bi-blends than in the monocultures.

Table 4. The dry matter produced by 48 seeds in two months.

MIXTURE*	DEPTH (mm)											
	10		30		60		90		120		150	
P	10.63	11.63	6.44	7.40	7.25	10.13	8.18	7.96	5.55	6.29	0.00	1.30
X	1.82	1.70	2.06	1.14	0.21	0.15	0.00	0.00	0.00	0.00	0.00	0.00
R	6.20	3.34	1.37	1.91	0.82	0.89	0.62	0.48	0.13	0.09	0.00	0.13
PX:P	5.54	3.92	6.29	6.86	5.73	4.25	4.74	4.54	1.46	4.42	0.00	0.00
PX:X	0.96	0.88	0.61	0.56	0.30	0.25	0.00	0.00	0.00	0.00	0.00	0.00
PR:P	4.08	4.31	4.95	4.26	6.12	6.25	2.69	3.73	2.71	2.34	0.00	0.00
PR:X	0.50	0.53	1.20	0.99	1.62	1.04	0.00	0.50	0.04	0.03	0.00	0.00
XR:X	0.92	0.64	0.65	0.84	0.48	0.46	0.14	0.00	0.00	0.00	0.00	0.00
XR:R	1.19	0.99	1.02	0.65	0.39	1.88	0.41	0.52	0.00	0.11	0.00	0.00
PXR:P	3.07	3.83	2.80	3.64	3.48	2.91	3.95	2.94	1.63	2.03	0.00	0.00
PXR:X	0.45	0.67	0.30	0.57	0.25	0.30	0.03	0.11	0.00	0.00	0.00	0.00
PXR:R	0.69	0.89	0.44	0.69	0.42	0.80	0.36	0.14	0.15	0.00	0.00	0.00

* P = *Paraserianthes lophantha*
X = *Acacia melanoxylon*
R = *A. mearnsii*

In monocultures, *A. mearnsii* establishes significantly more seedlings at 10 and 30 mm sowing depths than *P. lophantha*. At 90, 120 and 150 mm however, the roles are reversed and *P. lophantha* establishes significantly more seedlings than *A. mearnsii*.

Table 5. Significance levels (mean squares in the case of the error line) of a sequential analysis of variance of dry material yield for each of six depths (in mm). Parameters were fitted in the sequence shown. All significance levels less than 0.00005 are rounded up to 0.0001.

Source of variation	df	10	30	60	90	120	150
Blocks*	1	0.5066	0.5791	0.5760	0.9274	0.2684	0.4418
P	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0699
R	1	0.0008	0.0009	0.0130	0.1531	0.9637	0.7715
PR	1	0.0116	0.2703	0.0336	0.1245	0.5268	0.3124
X	1	0.0971	0.0006	0.2995	0.2559	0.9965	0.7438
PX	1	0.6036	0.0019	0.5440	0.1526	0.9905	0.5740
XR	1	0.2107	0.7343	0.3274	0.3482	0.9848	0.8889
PXR	1	0.4225	0.2920	0.4205	0.1018	0.9374	0.8729
Error	6	1.0598	0.3958	1.0258	0.3164	0.6364	0.1345

*P = *Paraserianthes lophantha*
X = *Acacia melanoxylon*
R = *A. mearnsii*

Table 6. Significance levels (mean squares in the case of the error line) of a sequential analysis of variance of dry material yield, by species, for each of six depths (in mm). Parameters were fitted in the sequence shown. All significance levels less than 0.00005 are rounded up to 0.0001.

Source of variation	df	10	30	60	90	120	150
Blocks*	1	0.4085	0.5067	0.5276	0.9209	0.2924	0.3011
P	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0222
R	1	0.0001	0.0001	0.0022	0.0220	0.7776	0.8328
PR:P	1	0.0573	0.6141	0.0136	0.0259	0.4076	0.0909
PR:R	1	0.0114	0.4278	0.2341	0.8087	0.9879	0.8331
X	1	0.0011	0.0001	0.2515	0.8100	1.0000	1.0000
PX:P	1	0.2122	0.0001	0.4246	0.0128	0.9745	0.2601
PX:X	1	0.8934	0.5183	0.8672	0.9833	1.0000	1.0000
XR:X	1	0.8400	0.8570	0.4838	0.7820	1.0000	1.0000
XR:R	1	0.0341	0.9644	0.2678	0.5416	0.9745	0.8458
PXR:P	1	0.2586	0.0708	0.2462	0.0066	0.9843	0.7104
PXR:X	1	0.9870	0.9743	0.9520	0.8542	1.0000	1.0000
PXR:R	1	0.5356	0.7629	0.6343	0.9633	0.9155	1.0000
Error	11	0.7155	0.2887	0.8425	0.2770	0.7739	0.0775

*P = *Paraserianthes lophantha*
X = *Acacia melanoxylon*
R = *A. mearnsii*

At 10 mm depth, *A. melanoxylon* establishes significantly more seedlings than *P. lophantha*, but not at 30 mm depth. From depths of 60 mm upwards *P. lophantha* establishes significantly more seedlings than *A. melanoxylon*. At 30, 60, 90 and 120 mm, *A. mearnsii* establishes significantly more seedlings than *A. melanoxylon* (Tables 1 and 2).

Dry matter production

No significant differences between dry matter production of species in tri-blends and in the *A. mearnsii/A. melanoxylon* bi-blends and the dry matter production of the respective species in monocultures are observed (Table 5). In the *P. lophantha/A. melanoxylon* biblend, significant differences only occur at the 30 mm depth (Table 5) where *P. lophantha* produces significantly more dry matter in the biblend than in the monoculture (Tables 4 and 6). In the *P. lophantha/A. mearnsii* biblend, significant differences occur at the 10 mm and 60 mm depths (Table 5). *A. mearnsii* production is significantly reduced in bi-blends at 10 mm depth while the production of *P. lophantha* is also reduced albeit not significantly (Table 4 and 6). At 60 mm depth the production of *P. lophantha* is significantly increased in the biblend.

5.4 Discussion

When grown in bi-blends at 10 mm sowing depths, *P. lophantha* and *A. melanoxylon* both produce significantly more seedlings than when grown in a biblend with *A. mearnsii*. *P. lophantha* also produces significantly less seedlings in bi-blends with *A. mearnsii* than in monocultures. These results indicate that *P. lophantha* and *A. melanoxylon* have positive interactions with one another but that both are negatively influenced by the presence of *A. mearnsii* in bi-blends. *A. mearnsii* therefore seems to outcompete *P. lophantha* and *A. melanoxylon* in the seedling phase. There is, however, no significant interaction when the three species are sown in a tri-blend. No biological explanation can be given for the apparent better competitive ability of *A. mearnsii*, except that pre-emergence interference, which may be of an allelopathic nature, occurs which limits the development of the other two species. No similar interference was found in the tri-blends. These interactions are therefore ascribed to variable germination at the 10 mm depth, a possible result of faster drying out of the soil at that depth than at the deeper sowing depths. *A. mearnsii* appears to

be more drought resistant than *A. melanoxylon* and *P. lophantha* during the germination phase (Chapter 3). Another possible reason for variable germination at the 10 mm depth is that the soil temperature in the glasshouse during sunny conditions could rise so high in the upper layers of soil that the imbibed seeds could have been damaged.

At 30 and 60 mm planting depths *A. mearnsii* and *A. melanoxylon* produce significantly more seedlings in bi-blends than in monocultures. At 60 mm the differences are more pronounced, especially in the case of *A. melanoxylon*. This trend is also reflected in the tri-blends, although differences are not statistically significant. The fact that *A. melanoxylon* produces more seedlings in bi-blends with *P. lophantha* and *A. mearnsii* is logical because *P. lophantha* and *A. mearnsii* are capable of establishing from greater depths. The positive effect that these two species have on the establishment of *A. melanoxylon* is probably a simple case of their more "powerful" seeds clearing a path through the soil thus making it easier for the less "powerful" *A. melanoxylon* seeds to reach the soil surface. The positive effect that *P. lophantha* has on the establishment of *A. mearnsii* can probably be explained in the same way. It is however difficult to explain the positive effect that the presence of *A. melanoxylon* seeds has on the establishment of *A. mearnsii* seeds. Because *A. melanoxylon* is the "weaker" seed, *A. mearnsii* should have been negatively influenced by its presence, especially at the deeper depths because the cumulative "emergence power" of a blend of these two species should be less than the "emergence power" of an *A. mearnsii* monoculture. If this phenomenon is not due to natural variation (which is denied by the significant statistical differences), then some unknown allelochemic effects might play a role and should be investigated further.

The behaviour of *A. mearnsii* in bi-blends at 30 and 60 mm are reflected again at 90 mm depth in both bi- and tri-blends. *A. mearnsii* again produces more seedlings in bi-blends with *P. lophantha* and *A. melanoxylon* than in monocultures, but it also produces significantly more seedlings in bi-blends with *P. lophantha* than in bi-blends with *A. melanoxylon*, which makes sense. At this depth, *P. lophantha* is beginning to be negatively influenced in bi-blends, especially bi-blends with *A. melanoxylon*, although no significant differences between bi-blends of *P. lophantha* with *A. melanoxylon* or *A. mearnsii* can be ascertained. The effect of *A. mearnsii* and *A.*

melanoxylon on *P. lophantha* is just the opposite from the effect of *P. lophantha* on *A. mearnsii* and *A. melanoxylon*. *P. lophantha* seeds in monoculture have a stronger "emergence power" than when mixed with *A. melanoxylon* or *A. mearnsii*, which have a weaker "emergence power" and therefore the "emergence power" of bi-blends would have been weaker than those of a *P. lophantha* monoculture.

At 120 mm the number of seedlings produced by *A. mearnsii* and *A. melanoxylon* is so small that no significant interactions could be ascertained. *P. lophantha*, however, again shows a more pronounced negative influence on emerging ability in bi-blends with *A. mearnsii* or *A. melanoxylon*. At 150 mm there is no interaction of any nature because no *A. melanoxylon* seedlings established and a negligible number of *A. mearnsii* and *P. lophantha* seedlings emerged.

A. mearnsii and *A. melanoxylon* produce more seedlings at the shallower sowing depths because most of the germinating seeds establish as seedlings. Although most of the germinating *P. lophantha* seeds probably establishes too, the difference in the number of seedlings that establish can probably be attributed to the lower percentage of viable seeds of *P. lophantha*; ± 65% for *P. lophantha* and ± 98% for both *A. melanoxylon* and *A. mearnsii* (Chapter 2).

The superior ability of *P. lophantha* seeds to produce seedlings from deeper sowing depths cancels out their lower viability at between 30 and 60 mm sowing depths. From 60 mm downwards, the ability to establish from deep sowing depths outweighs the disadvantage of lower viability. Table 1 shows that sowing depth only begins to influence establishment of *P. lophantha* seedlings at depths of 120 and 150 mm. *A. melanoxylon* appears to be sensitive to planting depth and is strongly influenced at 30 mm whereas *A. mearnsii* is more able to establish from deeper sowing depths. The fact that *A. mearnsii* has lighter seeds than *A. melanoxylon* is surprising in the light of their ability to establish from deeper sowing depths.

The superior ability of *P. lophantha* seeds to produce seedlings from deeper sowing depths and the better ability of *A. mearnsii* over *A. melanoxylon* are only partly explained by seed size. The mean seed mass of *P. lophantha*, *A. melanoxylon* and *A. mearnsii* respectively, are 68.4 mg, 17.0 mg and 15.9 mg (Chapter 2). Possible reasons for a seed's inability to establish from deeper sowing depths can be attributed

to either inability of seed to germinate at such depths, or to inability to grow through the soil layer to emerge due to a shortage of energy (Fenner 1985). However, enhanced ability to establish from deeper sowing depths in the presence of seeds of other species indicates that germination takes place at the deeper sowing depths. Eastin (1984) found that *Sesbania exaltata* seeds germinated at 13 cm depth, but did not reach the surface.

The larger seed size of *P. lophantha* is obviously beneficial in establishing from greater depths. Contrasting evidence regarding the relationship between seed size and ability to establish from deep germination exists. In studies where seeds of varying sizes within a species were compared, bigger seeds generally were able to establish seedlings from deeper sowing depths than small seeds (Black 1955, Fehr *et al.* 1973, Brecke & Duke 1980, Weller 1985, Wulff 1986, Hendrix & Trapp 1992). However, Beveridge and Wilsie (1959) and Qiu and Mosjidis (1993) found no correlation between seed weight and emergence from increased sowing depth of seeds. Seedling morphology could play a role e.g. in the case of onions (*Allium cepa*) there is a good correlation between seed weight and successful emergence from depth whereas the radish (*Raphanus sativa*) gives a poor correlation. The shape of the onion seedling is ideally formed to penetrate the soil whereas that of radish offers more resistance and the larger the cotyledon, the greater the resistance (Harper 1977).

Where seed sizes between species are compared, there is also no clear indication that bigger seeds always emerge from deeper depths. For instance, *Sesbania exaltata*, having smaller seeds than *Glycine max*, emerged from deeper sowing depths than the latter (Johnstone *et al.* 1979). This was also the case in this study where *A. mearnsii*, having the lighter seed, established from deeper sowing depths than *A. melanoxylon* (Table 1). A probable explanation is that the biochemical composition of *A. mearnsii* seeds is such that it has more energy per mass unit than *A. melanoxylon*. It has been shown that *A. mearnsii* seed contains a much higher oil content than the seed of *A. melanoxylon* (Chapter 2). Oil content is more important than carbohydrate and protein content as a source of energy reserves, because of its high relative energy content per unit of mass (Levin 1974).

Ecologically the differential ability to establish from varying sowing depths might be explained by the mode of dispersal of the two species. In Australia (the country of

origin of all three species), *Acacia* species are divided into species that are mainly ant-dispersed and species that are mainly bird-dispersed (O' Dowd & Gill 1986). Ant-dispersed species exhibit a small white or off-white funicle (seed stalk) which is attractive to ants and the seed are shed from the tree immediately after ripening. Ants quickly gather these seeds and bury them in their nests without affecting the seed dormancy. When soil temperatures rise, as a result of fires, the dormancy is broken and, provided enough moisture is available, the seed will germinate (Drake 1981).

Bird-dispersed species on the other hand, exhibit a large red, yellow or orange aril (seed stalk) which is attractive to birds and the seed are not shed after ripening but kept on the tree for a prolonged period in order to be available to birds feeding on the tree. The seeds pass through the digestive canal of the birds unharmed except that the dormancy of the seed is often broken by the digestive processes of the birds. After the now non-dormant seed is passed by the bird it will germinate if enough moisture is available (O' Dowd & Gill 1986).

It can therefore be reasoned that seeds adapted for bird dispersal will also be adapted for shallow germination and seeds adapted for ant dispersal will be better adapted for deeper germination. In this case *A. mearnsii* is adapted for ant dispersal while *A. melanoxylon* is adapted for bird dispersal (Davidson & Morton 1984, O' Dowd & Gill 1986).

Survival and early growth of seedlings are important parameters determining the outcome of competition between seedlings occurring in the same area (Harper 1977). Again, bigger seeds generally produce bigger seedlings that have a competitive advantage over smaller seedlings from smaller seeds, especially under adverse conditions (Black 1955, Black 1958, Beveridge & Wilsie 1959, Harper & Obeid 1967, Gross 1984, Stock *et al.* 1990, Peterson & Facelli 1992, Shanmuganathan & Benjamin 1992, Qiu & Mosjidis 1993 and Qiu *et al.* 1994). In many cases the bigger seedlings kept their advantage only under sub-optimal conditions, such as competition from existing vegetation (Gross 1984) or shaded conditions (Kolb *et al.* 1990). Seedling survival under low sunlight conditions is related to the amount of storage material available from the seed and the number and properties of extension sites in the shoot (Grime & Jeffery 1965). The early growth advantage of bigger seedlings over smaller seedlings was ascribed to bigger cotyledonary area (and not reserve content) of the

bigger seedlings (Black 1955) and hence superior light interception (Black 1958). Qiu *et al.* (1994), on the other hand ascribed better growth of seedlings from large seeds to the higher content of cotyledon reserves.

Hendrix *et al.* (1991) and Hendrix & Trapp (1992) however found that seedlings from smaller seeds survived drought conditions better due to a more favourable root length/total leaf area ratio. This advantage of the small seedlings however only lasted for about 20 days.

The effects that the three species under investigation had on one another regarding establishment in mixtures could be explained by the physical force of seedlings pushing through the soil together, as discussed earlier, or by some allelochemical interactions. Harper (1977) stated that groups of seedlings seem to break through capped soil more readily than isolated individuals - physical force seems to be needed.

These results could have some practical implications. If biocontrol agents can inhibit seed production of these plants significantly as has been shown for several of the *Acacia* species (Dennill & Donnelly 1991), seed density in the soil seed bank should decrease. Seeds that germinate singly will then be less able to establish from deeper depths than seeds germinating in groups. If seeds of *A. melanoxylon* for instance, are killed by a slow fire at depths >50 mm, it should not establish seedlings successfully if single seeds germinate at these depths. Reduction of seed rain can therefore lead to less dense seed banks and thus less successful establishment of seedlings from deeper buried seeds. Holmes (1989b) has shown that the median depth from which *A. cyclops* emerged after several burning treatments, was between 20 mm and 60 mm. On the other hand, Moran & Hoffmann (1989) have shown that reduction in seed production of *Sesbania punicea* led to bigger seed sizes, which will probably enhance the ability of seeds to emerge from depth. The effect of the reduced seed production on seed size has to the author's knowledge not been determined for any of the *Acacia* species under biocontrol.

P. lophantha produces more dry material than *A. mearnsii* or *A. melanoxylon* at all sowing depths as may have been expected considering seed size. The absence of definite trends at 10 mm is probably due to variation in germination and growth of the seedlings. A possible source of variation in germination and growth is the prevailing

moisture and temperature levels in the upper layers of the soil, as explained in the discussion dealing with the establishment of the seedlings. The absence of interactions in the 120 and 150 mm depths is probably due to the small number of seedlings that established.

The increases in dry matter production of *P. lophantha* in bi- and tri-blends are probably due to *P. lophantha* seedlings experiencing less inter-species competition from *A. melanoxylon* and *A. mearnsii* seedlings than intra-species competition from other *P. lophantha* seedlings in monocultures.

It is interesting to note that *A. mearnsii* and *A. melanoxylon*, but especially *A. melanoxylon*, do not produce significantly less dry matter in bi- and tri-blends when *P. lophantha* produces more dry matter in the same blends. This is probably due to the early termination of the experiment i.e. competition does not reach critical levels for *A. mearnsii* and *A. melanoxylon*, although *P. lophantha* benefits from the existing competition levels.

The fact that seedling densities in the pots vary because of varying numbers of emerging seedlings, excludes the opportunity to analyze competition effects between these species with the traditional methods used for replacement design experiments (De Witt 1960, Harper 1977). The delayed emergence of seedlings from deeper sowing depths would also have influenced the seedling size (Black & Wilkinson 1963). The conclusions drawn from the dry matter production of these experiments therefore can highlight certain trends which cannot be attributed with certainty to intra- or inter-specific competition. It certainly appears as if *P. lophantha* seedlings experience less competition from the two *Acacia* species than from their own seedlings.

The results confirmed the validity of the first hypothesis tested, *viz.* that *P. lophantha* seeds will establish from deeper sowing depths than the *Acacia* seeds. The second hypothesis however was refuted by the results. *A. mearnsii* seeds are superior to those of *A. melanoxylon* in respect of their ability to emerge from deeper sowing depths. The results only give rough indications of the competitive abilities of the seedlings. The varying numbers of seedlings establishing from different sowing depths should be taken into account when comparing the dry matter production of the

seedlings. It however appears as if *P. lophantha* will be the better competitor in the seedling stage, as is expected due to the larger seed size. *A. mearnsii* on the other hand, although being able to establish from deeper sowing depths than *A. melanoxylon*, did not appear to be superior to *A. melanoxylon* in terms of dry matter production after establishment of the seedlings. Competitive effects of these species in the seedling stage should be investigated further.

Chapter 6

Do seed characteristics, seed size in particular, influence seedling growth under varying nutrient concentrations, drought and shade conditions?

6.1 Introduction

Competition between co-occurring species can be severe in the seedling phase immediately after emergence. The establishment and subsequent growth rates are crucial factors determining the outcome of inter-specific competition (Harper 1977). Establishment and growth of seedlings takes place when the soil has a suitable particle size, compaction and aeration, pH values, available nutrient status and moisture values for successful root penetration and when the correct temperature, light, humidity and exposure to light are available for shoot and leaf growth (Crawley 1983). The success of a plant species depends on deficiency or excess with respect to any of several factors, such as heat, light, water or nutrients, which may approach the limits of tolerance for that species. Thus, plant species have an ecological minimum and maximum, with the range between these limits defining tolerance or ecological amplitude (Carter & Klinka 1992).

The tolerance levels of species to moisture, light and mineral nutrient stresses may give an indication of the habitat that can be invaded, as well as an indication of which species will be most successful under a given set of environmental resource conditions. In this experiment, *Acacia mearnsii* De Wild., *Acacia melanoxylon* R. Br. and *Paraserianthes lophantha* (Willd.) Benth. seedlings were grown in monocultures to determine their response to stress induced by varying levels of mineral nutrients, light and moisture. An attempt was also made to get preliminary information on how the species would react in competition with each other. A lack of greenhouse space prevented the use of a full-scale competition trial. Three hypotheses are tested in this study: i) *P. lophantha*, having the largest seed, and *A. melanoxylon*,

which is adapted to a forest environment, will be less influenced by shading than *A. mearnsii*; ii) *P. lophantha* and *A. mearnsii*, which are adapted to a more open habitat, will be more drought resistant than *A. melanoxylon*; and iii) *A. melanoxylon*, which has a higher nutrient content in its seed, will be less influenced by low nutrient conditions than the other two species.

6.2 Material & methods

Ripe seeds were harvested from *A. mearnsii, A. melanoxylon* and *P. lophantha* trees in the vicinity of Stellenbosch (33° 56' S; 18° 52' E) in December 1993. These were stored in glass jars with lids under cool, dark conditions until needed.

Greenhouse studies

Effect of different fertilizer concentrations on seedling growth

The experiment was conducted in a water-cooled glasshouse with night/day temperatures averaging about 15/30°C. Seeds of *A. mearnsii, A. melanoxylon* and *P. lophantha* were chipped at the distal end to break dormancy. Eight seeds of a species were sown into 7 *l* black nursery bags filled with sand on 27/12/1993. Seeds were sown in a 2X2 matrix resulting in a spacing of about 50 mm between seedlings. Additional seeds were sown in separate containers to replace seedlings that died in the early stages of the experiment, before mortality could be ascribed to seedling competition. Thirty-one days later, on 28/1/1994, seedlings were thinned to leave four seedlings per pot. To each of these sowing treatments, four fertilizer concentrations were added. Each treatment was replicated four times. A fertilizer solution containing 6.5% N, 2.7% P, 13.0% K, 7.0% Ca, 2.2% Mg, 7.5% S, 0.15% Fe, 0.024% B, 0.005% Zn, 0.002% Cu and 0.001% Mo was applied in one of four concentrations *viz.* full strength (100%), plus 75% , 50% and 25% of full strength. The fertilizer was applied through an automatic irrigation system where water was delivered into the container by means of thin plastic (spaghetti) tubing. Irrigation frequency depended on the amount of sunlight hours intercepted and was controlled by an automatic irrigation system (Sarnia Controls LTD, Guernsey, UK).

Seedlings were harvested 92 days after sowing (62 days after thinning) on 29/3/1994. Plants were cut at the soil surface and the length of the primary stem from

the soil surface to the apical leaf was measured. The total dry mass of the aboveground material was determined. The roots were washed under running water to remove the sand and the dry mass of the roots was determined as well as the length from the soil surface to the lowest apical root tip. In some cases, especially in the case of *P. lophantha*, roots from plants growing in the same container were so intermingled that it was difficult to separate them. Therefore some of the mass data of such plants may not reflect the exact amount of roots for each plant. Sand pebbles were also enveloped in the matted roots of *P. lophantha*, and in trying to remove them, plant material was sometimes lost. The roots of the *Acacia* species, on the other hand, were easy to wash without damaging them, because they were much smaller and did not occupy the whole volume of the container.

To determine the effects of fertilizer concentration on the growth of each of the three species in monoculture, the following analysis was done. Experimental design was a 3 X 4 Completely Randomized Block design (CRBD) with four replicates using the factors Species (*A. mearnsii, A. melanoxylon* and *P. lophantha*) and Fertilizer (100%, 75%, 50% and 25%). Treatment means for root and shoot length and root and shoot mass were calculated using the LSMEANS statement of PROC GLM (SAS Institute Inc. 1985).

In addition, a preliminary competition experiment was performed by sowing seeds of the species in 0:4, 1:3, 2:2, 3:1 and 4:0 mixtures in a replacement series design for all species combinations to investigate competition effects. Lack of greenhouse space prevented the investigation of different sowing densities, therefore only four seeds per container were sown in the manner explained above. The mixture treatments were replicated twice and the same nutrient treatments as described above were applied to the mixtures. The analysis was done by comparing species by means of a 4:0, 2:2, and 0:4 replacement design (due to excessive mortality of especially the *A. mearnsii* seedlings, the 3:1 and 1:3 mixture treatments were terminated during the course of the experiment). The results of the replacement experiment were interpreted by calculating the relative yield total (RYT) and the relative crowding coefficient (K) (Harper 1977; Bi & Turvey 1994). The relative yield total estimates whether the two

species are making demands on the same resources, *i.e.* the degree of overlap in the use of resources:

$$RYT = Y_{ij} / Y_{ii} + Y_{ji} / Y_{jj} \quad (1)$$

where Y_{ji} is the yield of species J mixed with species I and Y_{jj} is the yield of species J in a monoculture. A value of RYT of 1.0 implies that the two species are making demands on the same limiting resources of the environment. Values of RYT > 1.0 suggests that, although the species may still be competing for the same resources, they also make demands on different resources. Values of RYT < 1.0 indicate a mutual antagonism.

The relative crowding coefficient is a formal measure of the aggressiveness of one species towards another. It takes the following form:

$$K_{ij} = F_j / (Y_{ii} - Y_{ij}) F_i \quad (2)$$

where K_{ij} is the relative crowding coefficient of species I towards species J, Y_{ii} is the yield of species I in its monoculture, and Y_{ij} the yield of species I in mixture with species J, the relative frequencies of the species being F_j and F_i .

Joliffe *et al.* (1984) calculated two indices indicating the relative effects of intra- and inter-specific competition:

$$R_{ii} = (Y_{ip} - Y_{ii}) / Y_{ip} \text{ and } R_{ji} = (Y_{ii} - Y_{ij}) / Y_{ii} \quad (3)$$

where R_{ii} is the relative effect of intra-specific competition on plants of species I and R_{ji} is the relative effect of inter-specific competition from species J on the yield of species I. Y_{ip} is the "projected yield" of species I, which is the expected yield of that species at a given density in the absence of intra- and inter-specific competition. It can be calculated as the density of the species times the yield of the species when grown individually free from competition. Although R_{ii} and R_{ji} should both be known to give a meaningful indication of the effect of crowding on the yield of a species, in this experiment no data on the effect of intra-specific competition was available because no variation in the density of the monocultures was included in the experimental layout. However, the R_{ji} values of different species towards each other will give an indication of the relative aggressiveness of two species involved in a replacement design experiment.

The replacement series competition experiments first described by De Witt (1960), have been criticised recently as being statistically invalid and hard to interpret (Snaydon 1991, 1994). Replacement series experiments are usually carried out at a single density and as a result, the effects of intra-specific competition cannot be isolated from those of inter-specific competition (Firbank & Watkinson 1985). As a result, RYT- values cannot be used as an accurate measure of resource complementary in replacement designs (Snaydon & Satorre 1989). Joliffe *et al.* (1984), however, stated that a major strength of the replacement series approach is that the effects of changing species proportions can be examined at a constant total planting density. Austin *et al.* (1988) and Law and Watkinson (1987) stressed the importance of analyses of competition based on a wide range of densities of both interacting species. Sackville-Hamilton (1994) defended the replacement series designs by pointing out that additive designs have similar problems. He concluded that additive designs are appropriate for quantifying inter-component competition regardless of intra-component component competition. Replacement designs are appropriate for questions based on the similarity of competing taxa and the relationship of inter- to intra-component competition. From the preceding discussion it is clear that no conclusive evidence exists that can rule either additive or replacement designs incorrect.

In this experiment the RYT-, K- and R-values of the three species in different combinations were calculated for root mass and shoot mass. These values were not analyzed statistically due to problems with the statistical analysis of replacement design experiments (Snaydon 1991).

Effect of shade and drought stress on seedling growth

An experiment with a similar experimental design to the one described above was performed in a greenhouse under similar conditions. Seed of the same species as used above were sown in the same monoculture and mixture combinations as described above. These plants were subjected to three moisture and three light intensity levels during the growth phase. In this case however, in order not to confuse effects of water and fertilizer, fertilizer was applied beforehand by mixing controlled release pellets containing 15% N, 4.2% P and 10% K with the potting soil. A commercial mixture of

micro-elements was sprayed onto the plants at fortnightly intervals. Irrigation took place via the same system described in the previous experiment, except that in this case pure water was applied. A water gradient was provided by irrigating the one treatment normally, the next treatment every second day and the third treatment every fifth day by means of the irrigation system. The plants therefore received the same amounts of water on each watering day. The irrigation treatments were rated as having moisture levels of 100% (irrigated every day), 50% (irrigated every second day) and 20% (irrigated every fifth day). The lower water levels did not influence germination and establishment of seedlings. Light intensity was manipulated by enclosing the seedlings in 1.5 m tall shade cloth cages of different densities. The light intensity levels consisted of a 100% sunlight treatment (control), a 60% sunlight and a 20% sunlight treatment.

Seeds were sown on 31/12/1993 and were thinned to four plants per pot as described in the previous experiment after 32 days on 2/2/1994. The seedlings were harvested 95 days after sowing (62 days after thinning) on 5/4/1994. Shoot data were determined as described before. Due to problems with the collection of root data as discussed in the previous section, root data were not collected for this experiment. Experimental design was a 3 X 3 X 3 CRBD with four replicates and factors namely: Species (the three species mentioned), Moisture (100%, 50% and 20%) and Sunlight (100%, 60% and 20%).

Two analyses as described for the first experiment above were performed on the data. In the first analysis the effect of different moisture and shade levels on the growth of monocultures were analyzed. Treatment means for shoot length and shoot mass were calculated using the LSMEANS statement of PROC GLM (SAS Institute Inc. 1985).

Secondly, the RYT-, K- and R-values were calculated as described in the previous experiment, but in this experiment enough seedlings survived to be able to include the full 4:0, 3:1, 2:2, 1:3 and 0:4 replacement series.

6.3 Results

Effect of fertilizer concentration

Effect on monocultures of the three species

Significant interactions occur between species and fertilizer in terms of shoot mass, shoot length and root mass (P=0.0001, P=0.0192 and P=0.0001 respectively). Root length, however shows no interactions, but significant differences occur between species (P=0.0001) and between different levels of fertilizer (P=0.0016).

Shoot mass, shoot length and root mass generally show similar trends (Figure 1a,b,c). *P. lophantha* shows an inexplicable yield reduction when the fertilizer concentration is increased from 25% to 50%, but the highest fertilizer concentration generally gives the best yield, except in the case of root mass. The problems with respect to the determination of root mass have been discussed in the previous section and can possibly explain the slight drop in root mass at the 100% fertilizer level. The two *Acacia* species show mild reactions to fertilizer level and the most noteworthy trend here is the yield reduction of *A. mearnsii* at the highest fertilizer concentration. The root length data is probably a misrepresentation of what would happen in nature because roots of especially *P. lophantha* quickly reached the maximum length that the pot would allow. The erratic response of root length to fertilizer level (Figure 2a) could be explained by this problem. *P. lophantha*, as expected, produces the longest roots, while *A. melanoxylon* also produces significantly longer roots than *A. mearnsii* (Figure 2b).

Effect on competition

A. mearnsii/A. melanoxylon

Root- and shoot competition between these two species appear to follow the same trends and will therefore be discussed simultaneously (Figure 3a-h). Two peculiar features are the yield reduction of the *A. mearnsii* monoculture relative to the yield in combination with *A. melanoxylon* at the 50% fertilizer level (Figure 3c,d) and the apparent negative effects that both species exert on one another at the 75% fertilizer level (Figure 3e,f).

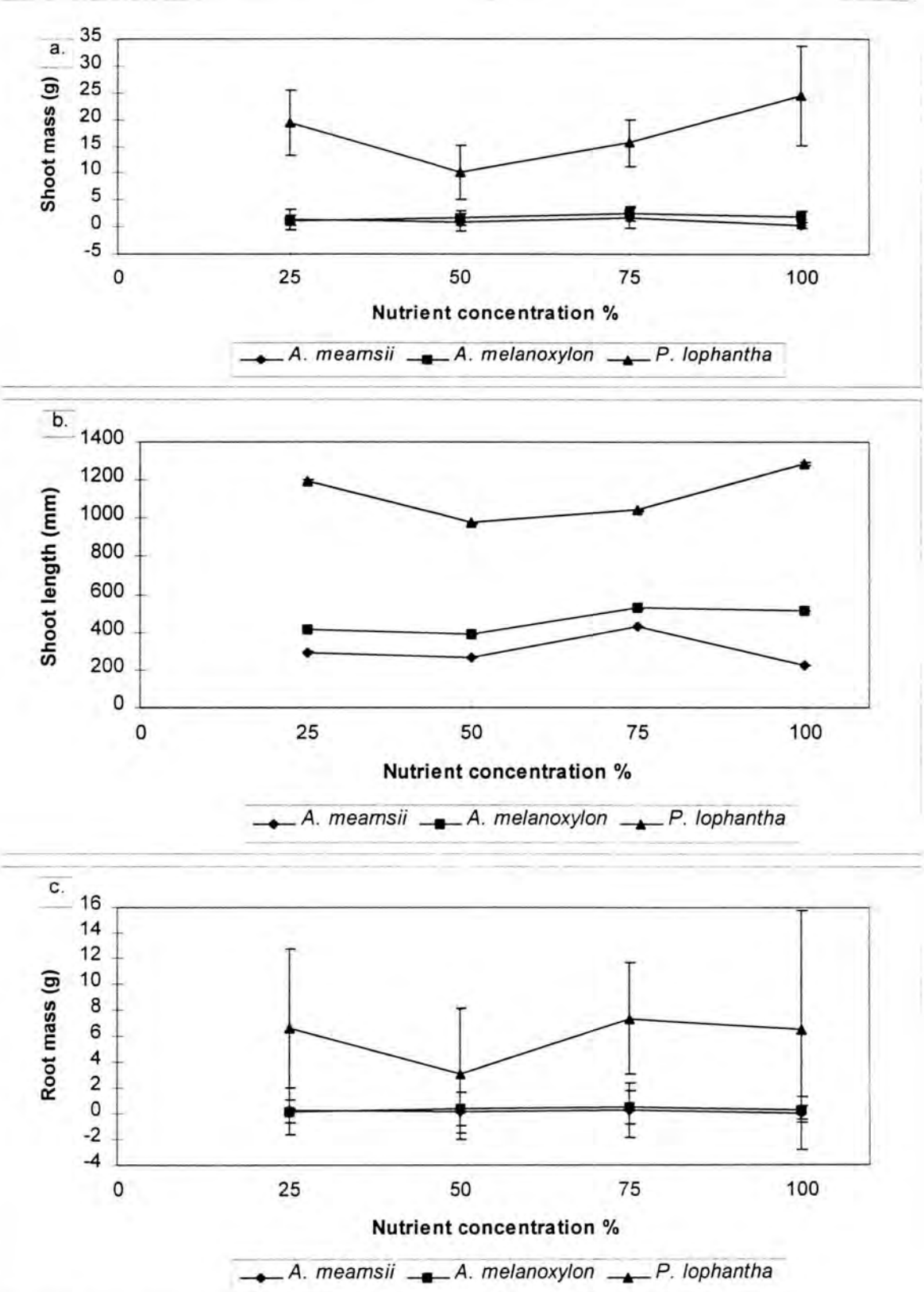

Figure 1. The interaction between three species (*Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha*) and nutrient concentration in terms of: **a**. mean shoot dry mass; **b**. mean shoot length and; **c**. mean root dry mass. (Vertical bars indicate standard deviation).

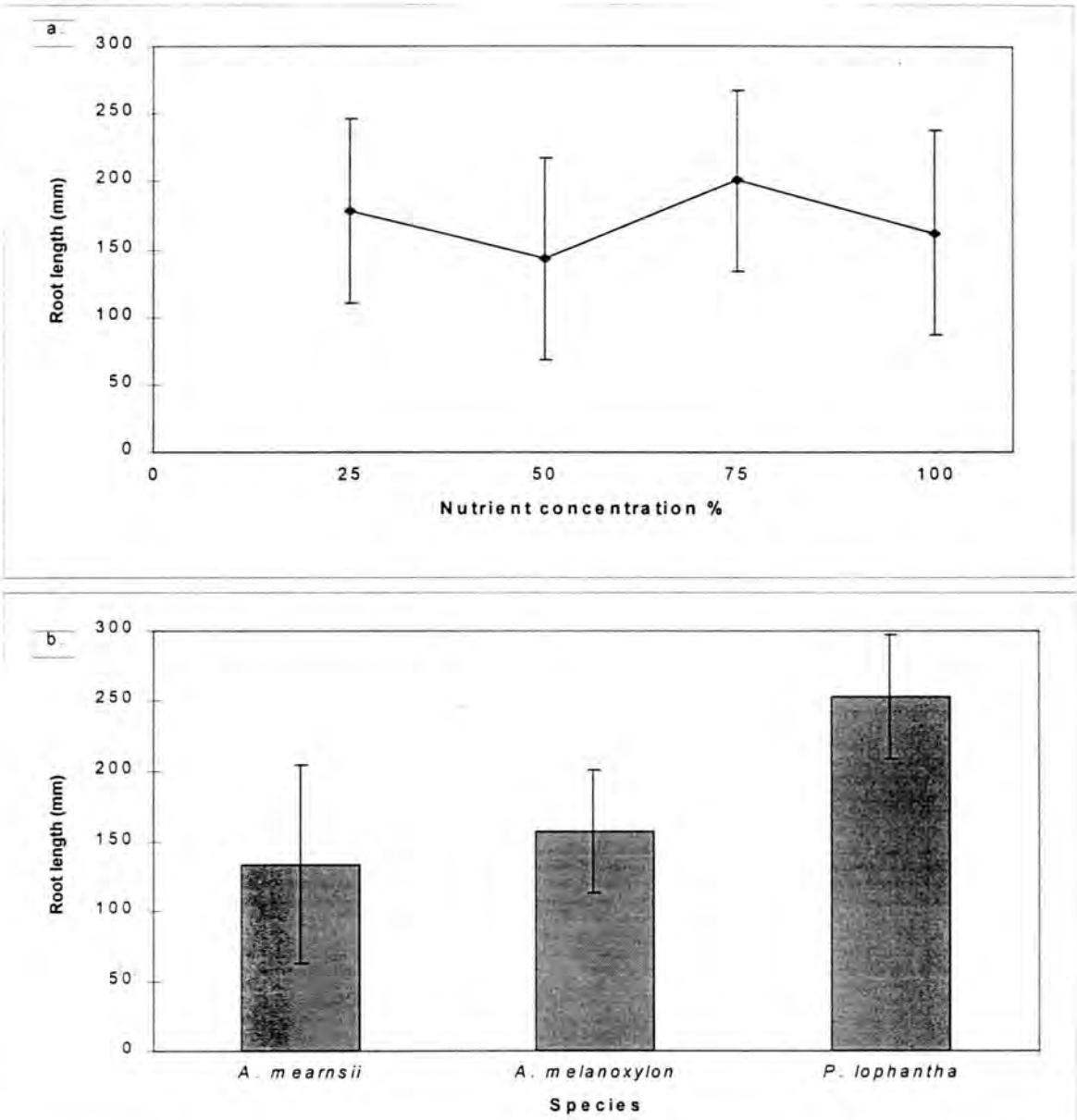

Figure 2. **a**. The effect of nutrient concentration on the mean root length of *Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*. **b**. The mean root length of *A. mearnsii*, *A. melanoxylon* and *P. lophantha* over all nutrient concentrations. (Vertical bars indicate standard deviation).

These phenomena are reflected in the RYT-values for the respective mixtures. At the 25%, 50% and 100% fertilizer levels *A. mearnsii* is not negatively influenced by *A. melanoxylon* judging from the convex shape of the curve. At the two lowest fertilizer levels *A. melanoxylon* is also not influenced by *A. mearnsii*, but at the 100% fertilizer level the slightly concave curve of *A. melanoxylon* shows that it was negatively influenced by the presence of *A. mearnsii*.

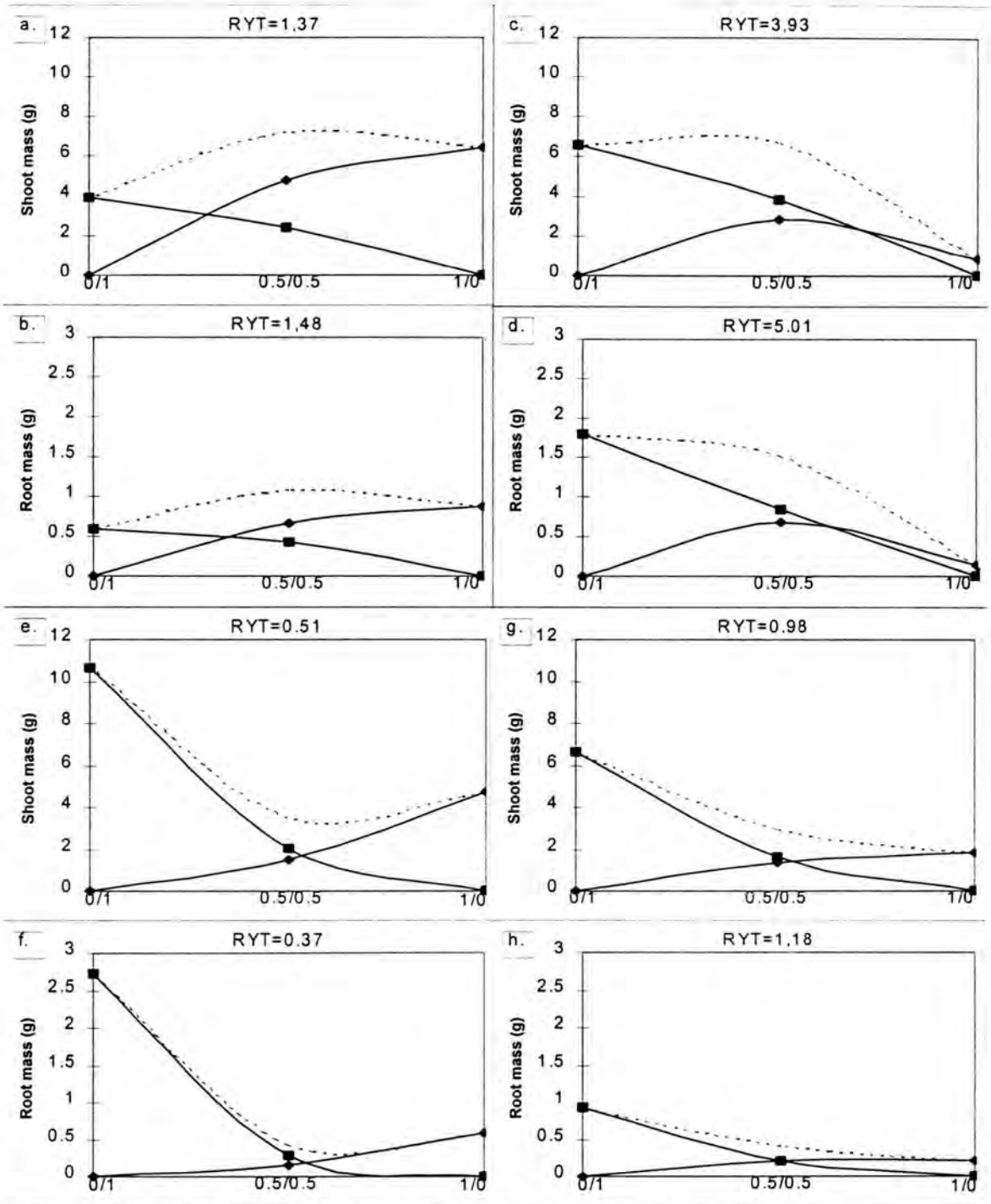

Figure 3. Replacement diagrams for *Acacia mearnsii* and *A. melanoxylon* grown in monocultures and 1:1 mixtures at four nutrient concentrations (**a**, **c**, **e**, and **g** = shoot mass totals for the 25%, 50%, 75% and 100% nutrient concentrations respectively; **b**, **d**, **f** and **h** = root mass totals for the 25%, 50%, 75% and 100% nutrient concentrations respectively. ◆ = *A. mearnsii* and ■ = *A. melanoxylon.* The dotted line indicates the total pot yield.).

Table 1. The relative crowding coefficient (K) of species in mixtures under different nutrient levels (a = *Acacia mearnsii*, l = *A. melanoxylon* and p = *Paraserianthes lophantha*).

Fertilizer concentration	Shoot	K_{al}	K_{la}	K_{lp}	K_{pl}	K_{ap}	K_{pa}
25%		2.98	1.62	0.63	2.32	0.29	1.31
50%		-1.43	1.46	0.67	5.85	0.3	1.75
75%		0.46	0.24	0.31	2.86	0.65	-3.39
100%		2.79	0.32	0.31	3.03	1.47	3.78
	Root						
25%		3	2.69	2.12	2.86	0.16	1.05
50%		-1.28	0.89	0.44	-3.45	0.14	-2.89
75%		0.37	0.12	0.43	0.96	-0.11	0.61
100%		20	0.29	0.51	3.12	0.63	3.72

Table 2. The relative effect of inter-specific competition (R) of species in mixtures under different nutrient levels (a = *Acacia mearnsii*, l = *A. melanoxylon* and p = *Paraserianthes lophantha*).

Fertilizer concentration	Shoot	R_{al}	R_{la}	R_{lp}	R_{pl}	R_{ap}	R_{pa}
25%		0.38	0.25	0.3	0.62	0.43	0.78
50%		0.41	-2.34	0.15	0.6	0.36	0.77
75%		0.81	0.68	0.26	0.76	-0.11	0.61
100%		0.76	0.26	0.25	0.76	0.21	0.41
	Root						
25%		0.27	0.25	0.26	0.32	0.49	0.86
50%		0.53	-3.53	-0.41	0.69	-0.53	0.88
75%		0.9	0.73	0.51	0.7	-0.42	0.76
100%		0.77	0.05	0.24	0.66	0.21	0.62

The K- and R-values also indicate that the presence of *A. mearnsii* in the mixture has a more pronounced effect on the yield of *A. melanoxylon* than *vice versa* (Table 1, Table 2). The crowding coefficient of *A. mearnsii* towards *A. melanoxylon* (K_{al}) and the relative effect of inter-specific competition of *A. mearnsii* on *A. melanoxylon* (R_{al}) are generally bigger than the K_{la} and R_{la} values.

The negative effect that these two species have on each other at the 75% level, could possibly be explained by the fact that it is the level where *A. melanoxylon* produced the biggest and *A. mearnsii* the second biggest yield. It has been shown that the fiercest competition occurs under the most fertile conditions where optimum growth occurs (Bazzaz & Harper 1976). Both species show optimum or near optimum growth at the 75% level and therefore the competition at that level could be severe. The reduction in yield of *A. mearnsii* in monoculture relative to its yield in the mixture (Figure 3c,d) might indicate that intra-specific competition in *A. mearnsii* is more severe than inter-specific competition from *A. melanoxylon*. However, the trend is not repeated at the other levels, and it is probably a matter of the exceptional high variability in growth of *A. mearnsii* seedlings under glasshouse conditions which is the cause of the yield reduction. Considerable variation in the yield of individual seedlings in the same pot where plants were grown in monoculture occurred in all species, but the variation in *A. mearnsii* was exceptional. Usually, when two or more *A. melanoxylon* or *P. lophantha* seedlings in a pot remained small, the remainder attained a reasonable size, probably a result of intra-specific competition. In the case of *A. mearnsii*, however, often all four plants would remain small while in some cases one, two or even three plants would grow much bigger than the rest. The inconsistency of the occurrence of big plants in *A. mearnsii* monocultures leads one to believe that the variation in seedling size is due to something other than intra-specific competition. It is perhaps genetic variation which causes the erratic growth of *A. mearnsii* seedlings in the glasshouse. *A. mearnsii* seedlings suffered the highest mortality percentage in the early stages of this experiment, before mortality could be ascribed to competition (10.4% *vs.* 4.6% and 0% of *A. melanoxylon* and *P. lophantha* respectively).

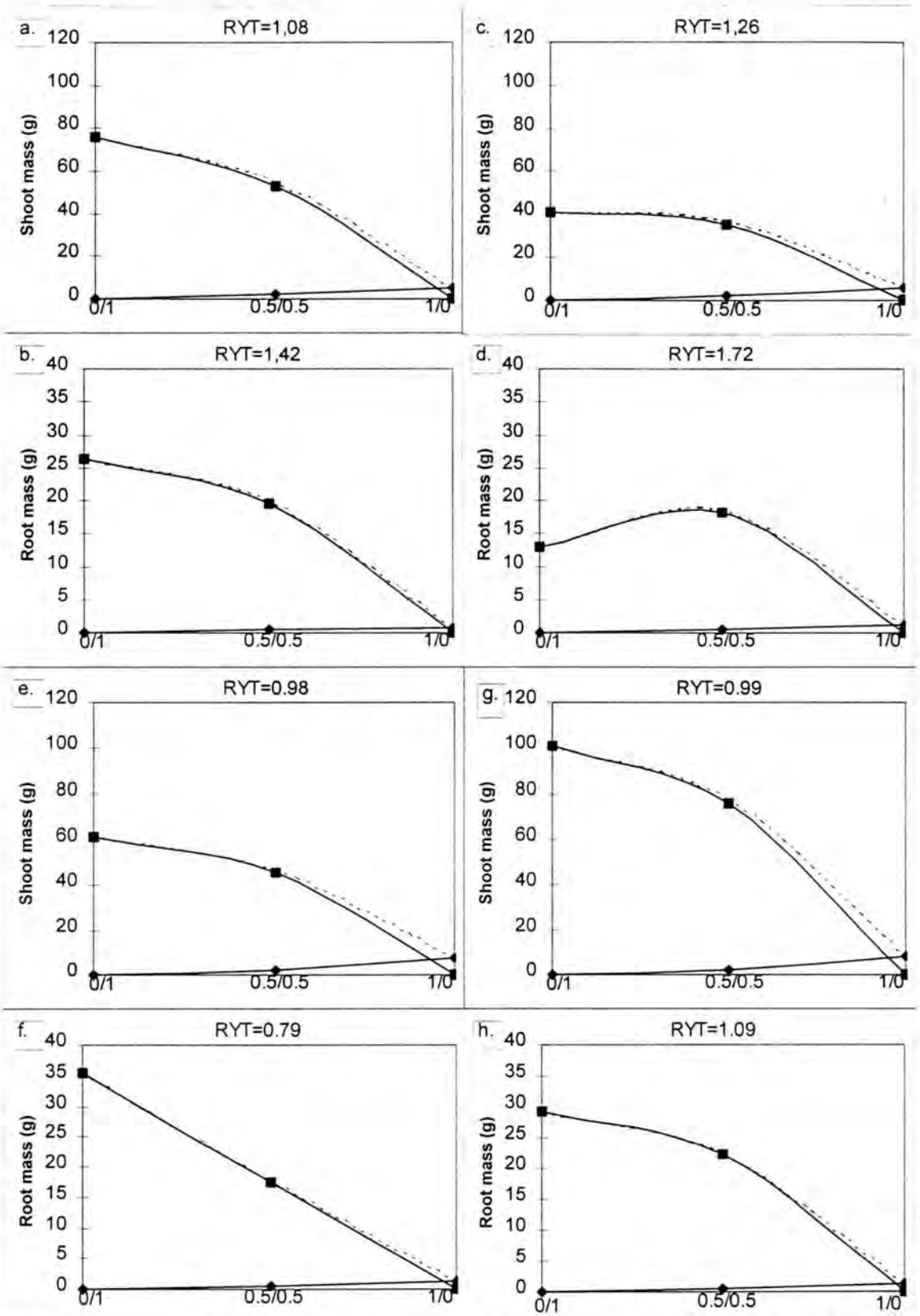

Figure 4. Replacement diagrams for *Acacia melanoxylon* and *Paraserianthes lophantha* grown in monocultures and 1:1 mixtures at four nutrient concentrations (**a**, **c**, **e**, and **g** = shoot mass totals for the 25%, 50%, 75% and 100% nutrient concentrations respectively; **b**, **d**, **f** and **h** = root mass totals for the 25%, 50%, 75% and 100% nutrient concentrations respectively. ◆ = *A. melanoxylon* and ■ = *P. lophantha*. The dotted line indicates the total pot yield.).

A. melanoxylon/P. lophantha

Again not enough differences occur between the root- and shoot data to warrant separate discussion of the two variables. In all cases *P. lophantha* appears to have a greater negative effect on *A. melanoxylon* than *vice versa* (note the convex form of the *P. lophantha* curves and the concave form of the *A. melanoxylon* curves) (Figure 4a-h). The RYT values suggest that the two species generally compete for the same resources. The K_{pl} and R_{pl} values are consistently higher than the corresponding K_{lp} and R_{lp} values, except for the root values at 50%, where negative values are evident (Table 1, Table 2). Negative K_{ij} values can be interpreted as that species i growsbetter in a mixture with species j than in a monoculture. Negative R_{ji} values would indicate the same. Possible explanations could be that species j has a positive allelopathic influence on species i or that intra-specific competition effects from species i on itself is more severe than inter-specific competition effects from species j on species i. It can therefore be concluded that *P. lophantha* is a superior competitor to *A. melanoxylon* under all fertilizer levels.

A. mearnsii/P. lophantha

Figure 5a-h indicates that, under all fertilizer levels, *P. lophantha* is a better competitor than *A. mearnsii*, especially relating to root growth. Table 1 shows that K_{pa} is consistently higher than K_{ap}, the negative values of K_{pa} indicate an even greater influence by *P. lophantha* on *A. mearnsii* than is simply indicated by a bigger K_{pa} value (see discussion of the *A. melanoxylon/P. lophantha* section above). Table 2 shows the same trend for R-values. In this case the negative R_{ap} values have the same meaning as negative K_{pa} values. It can therefore be concluded that *P. lophantha* outcompetes *A. mearnsii* under all fertilizer levels.

Effect of shade and drought stress on seedling growth

Effect on monocultures of the three species

The analysis shows a three way interaction between species, moisture and sunlight for shoot dry mass and shoot length (P=0.0110 and P=0.0062 respectively). The shoot mass yield of *P. lophantha* reacts positively to increases in the available moisture and sunlight, except in the case of 60% sunlight at 100% moisture, but the reduction in

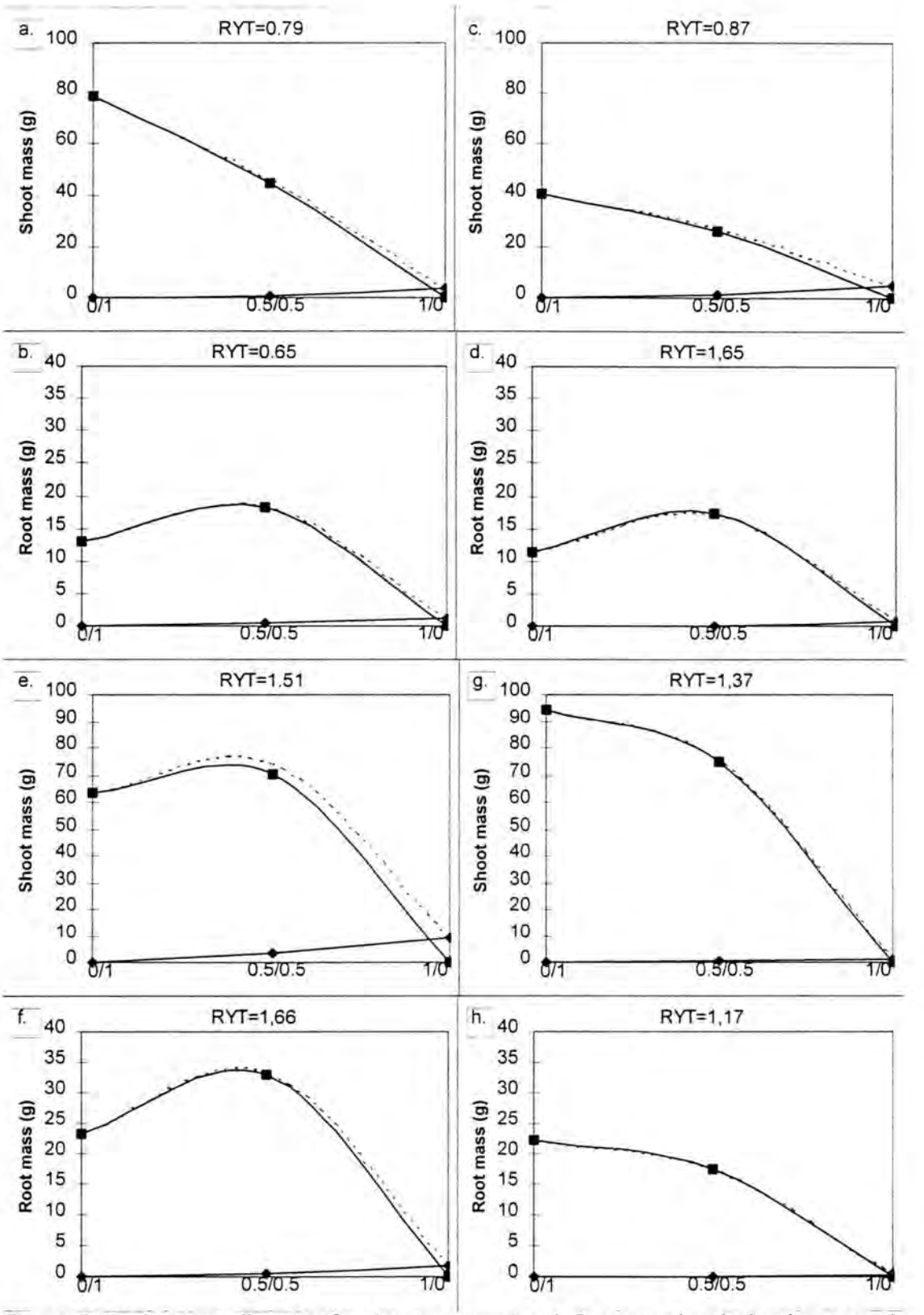

Figure 5. Replacement diagrams for *Acacia mearnsii* and *Paraserianthes lophantha* grown in monocultures and 1:1 mixtures at four nutrient concentrations (**a**, **c**, **e**, and **g** = shoot mass totals for the 25%, 50%, 75% and 100% nutrient concentrations respectively; **b**, **d**, **f** and **h** = root mass totals for the 25%, 50%, 75% and 100% nutrient concentrations respectively. ◆ = *A. mearnsii* and ■ = *P. lophantha*. The dotted line indicates the total pot yield.).

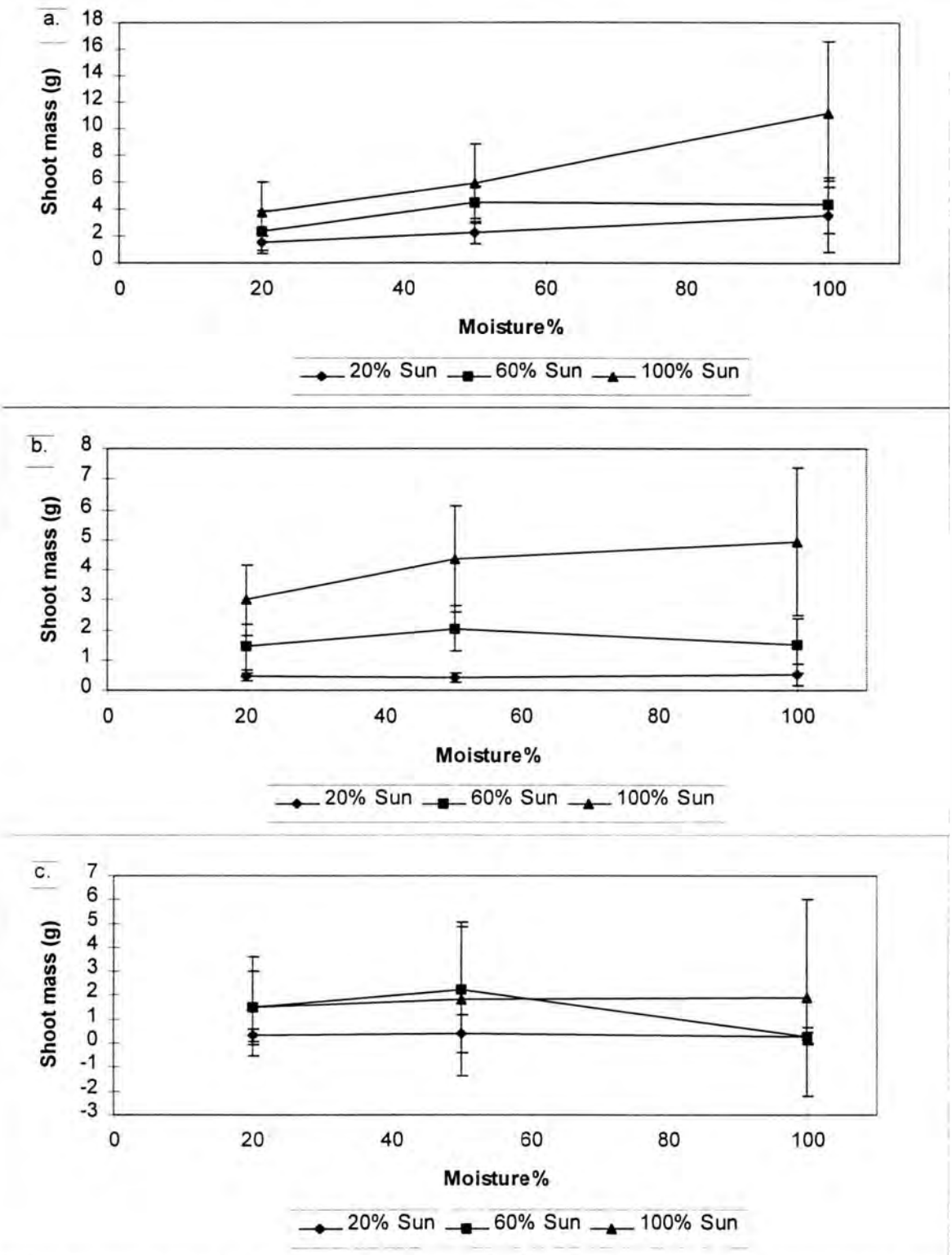

Figure 6. The interaction between moisture level and sunlight level for mean shoot mass of: **a.** *Paraserianthes lophantha*; **b.** *Acacia melanoxylon* and **c.** *A. mearnsii.* (Vertical bars indicate standard deviation).

growth is so small that it can be ignored (Figure 6a). *A. melanoxylon* shoot mass follows the same trend at 60% and 100% sunlight. The 20% sunlight treatment does not respond to moisture addition and shows that *A. melanoxylon* is sensitive to heavy shading and that light was the factor limiting growth in that treatment combination (Figure 6b). *A. mearnsii* exhibits the same trend at the 60% sunlight level, whilst the 20% sunlight and 100% sunlight levels do not respond to increased moisture (Figure 6c). This again indicates that light is the limiting factor in the 20% sunlight treatment. Why the plants did not respond to increased water levels at the 100% sunlight treatment, is hard to explain. In both the 60% and 100% sunlight treatments some growth increase occurred from the 20% to 50% moisture level, but it was not repeated when the moisture level was elevated to 100%.

The shoot length of *P. lophantha* generally follows the trends shown by the shoot mass (Figure 7a). The differences between treatments (especially light treatments), however, are smaller, indicating the greater plasticity of longitudinal growth compared to mass yield in reaction to shade. The shoot length of *A. melanoxylon* follows the shoot mass trend at 20% sunlight, but differs at the 60% sunlight treatment. No reduction in longitudinal growth from the 50 to 100% level, as is observed for shoot mass, occurs (Figure 7b). This again is an indication of plasticity in longitudinal growth, because the lower mass growth rate is not reflected in the longitudinal growth. The reduction in longitudinal growth at the 100% sunlight level from the 50% to 100% moisture level might be due to an oversupply of water. The mass increase at this level (Figure 6b) is probably the result of more growth being channelled into secondary and tertiary shoots, rather than into the leader shoot, which indicates longitudinal growth. The general trend for longitudinal growth is the same as for mass growth for *A. mearnsii*, with the exception of the 20% sunlight level at the 20% moisture level, where the plants grow taller than at the other moisture levels (Figure 7c).

Competition effects

A. mearnsii/A. melanoxylon

A. melanoxylon appears to be the more aggressive grower at the 100% moisture level, irrespective of sunlight level, although the monoculture of *A. melanoxylon* is

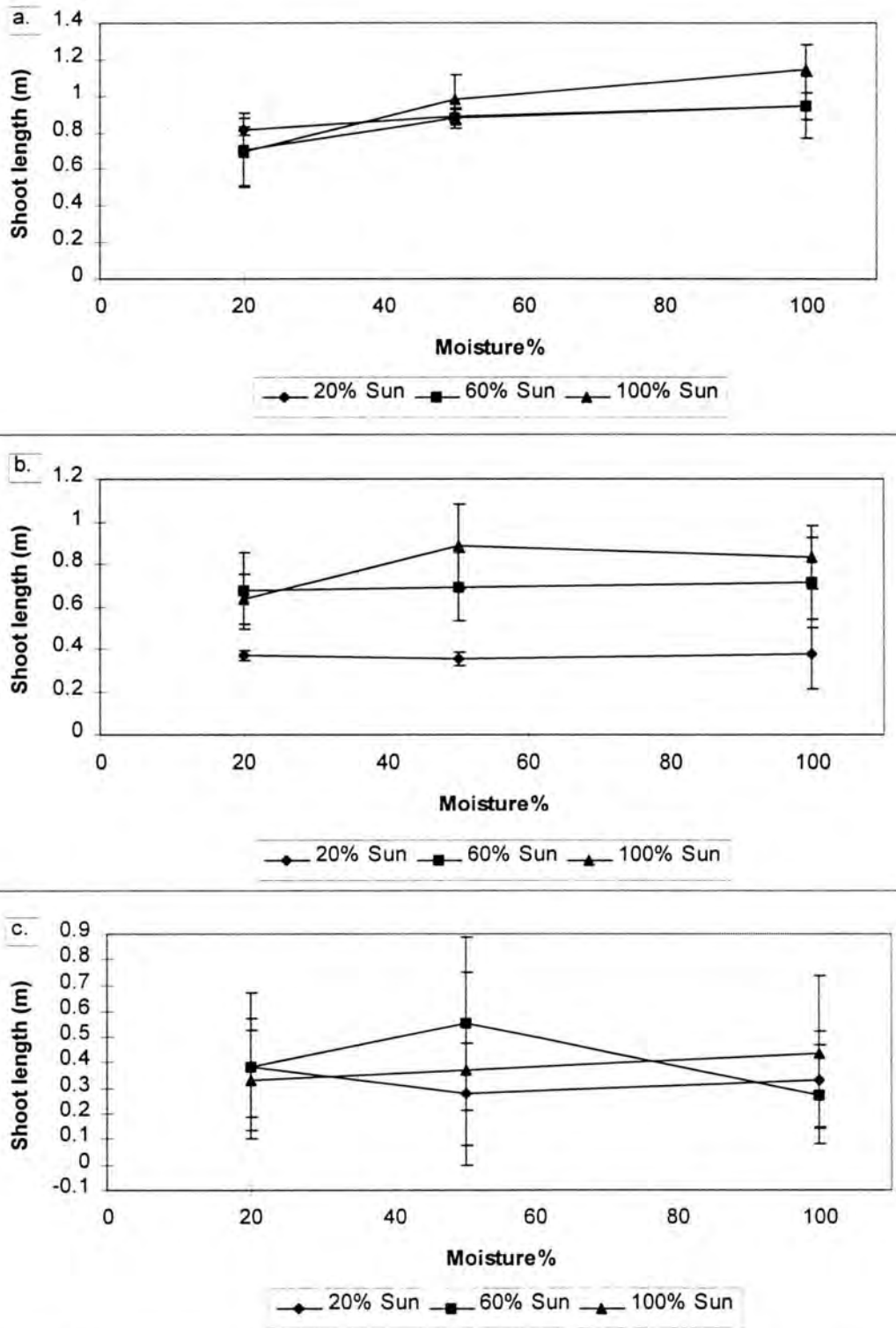

Figure 7. The interaction between moisture level and sunlight level for mean shoot length of: **a**. *Paraserianthes lophantha*; **b**. *Acacia melanoxylon* and **c**. *A. mearnsii.* (Vertical bars indicate standard deviation).

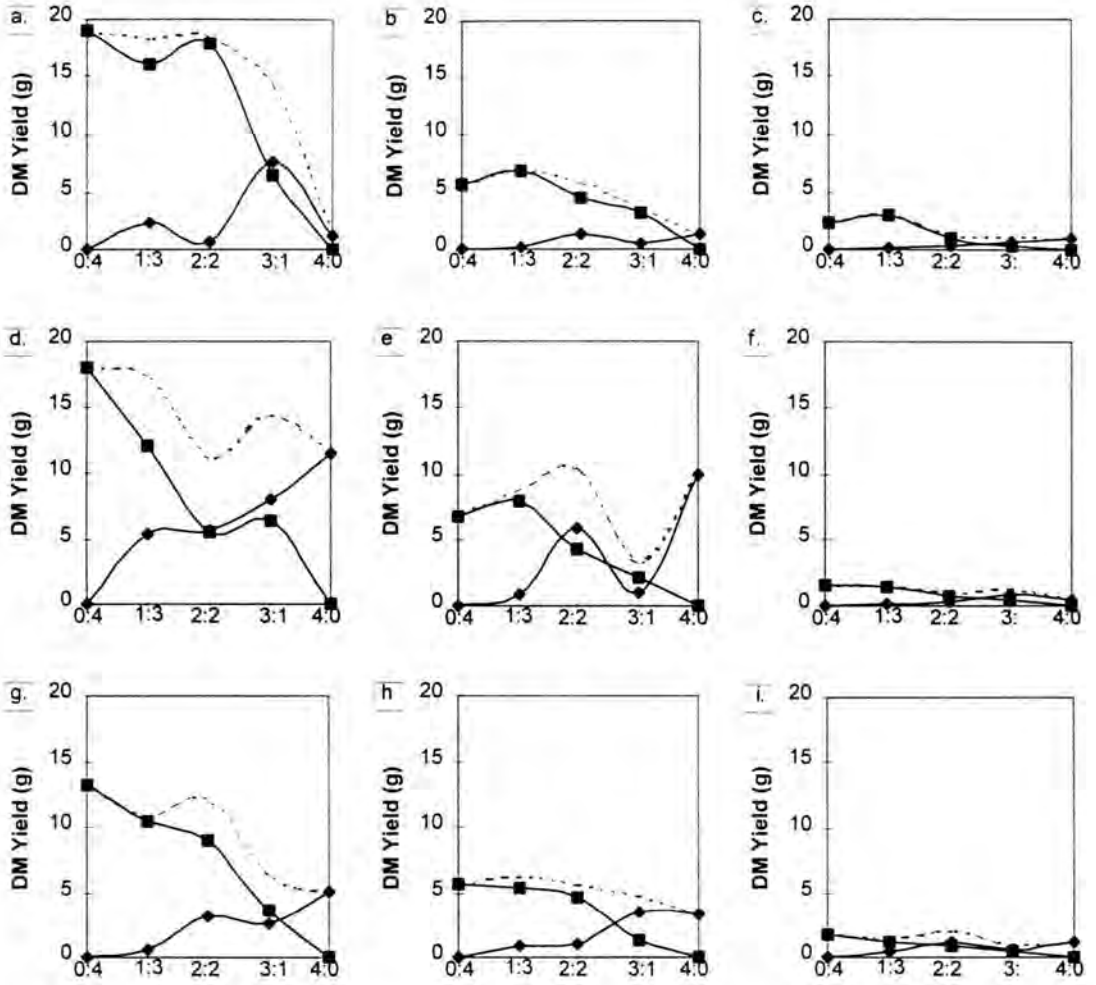

Figure 8. Replacement diagrams (total shoot mass) for *Acacia mearnsii* and *A. melanoxylon* grown in monoculture and in 1:1 mixtures under varying moisture/sunlight levels (Moisture/sunlight levels (%) are as follows: **a** = 100/100; **b** = 100/60; **c** = 100/20; **d** = 50/100; **e** = 50/60; **f** = 50/20; **g** = 20/100; **h** = 20/60; **i** = 20/20. ◆ = *A. mearnsii* and ■ = *A. melanoxylon*. The dotted line indicates the total pot yield.).

influenced to a greater extent by low light levels than is the monoculture of *A. mearnsii* (Figure 8a,b,c). At the 50% moisture level *A. mearnsii* appears to compete better with *A. melanoxylon* and is also influenced to a greater extent by low light levels than at the 100% moisture levels (Figure 8 d,e,f). At the 20% moisture level the two species appear to compete evenly, although *A. melanoxylon* produces a slightly higher yield in monoculture than *A. mearnsii*. Both species are negatively influenced by the lower light levels, *A. melanoxylon* more so than *A. mearnsii*. The RYT-values show no trend whatsoever, and it is therefore impossible to determine to which extent the two species share the same resources (Table 3). The R- and K-values

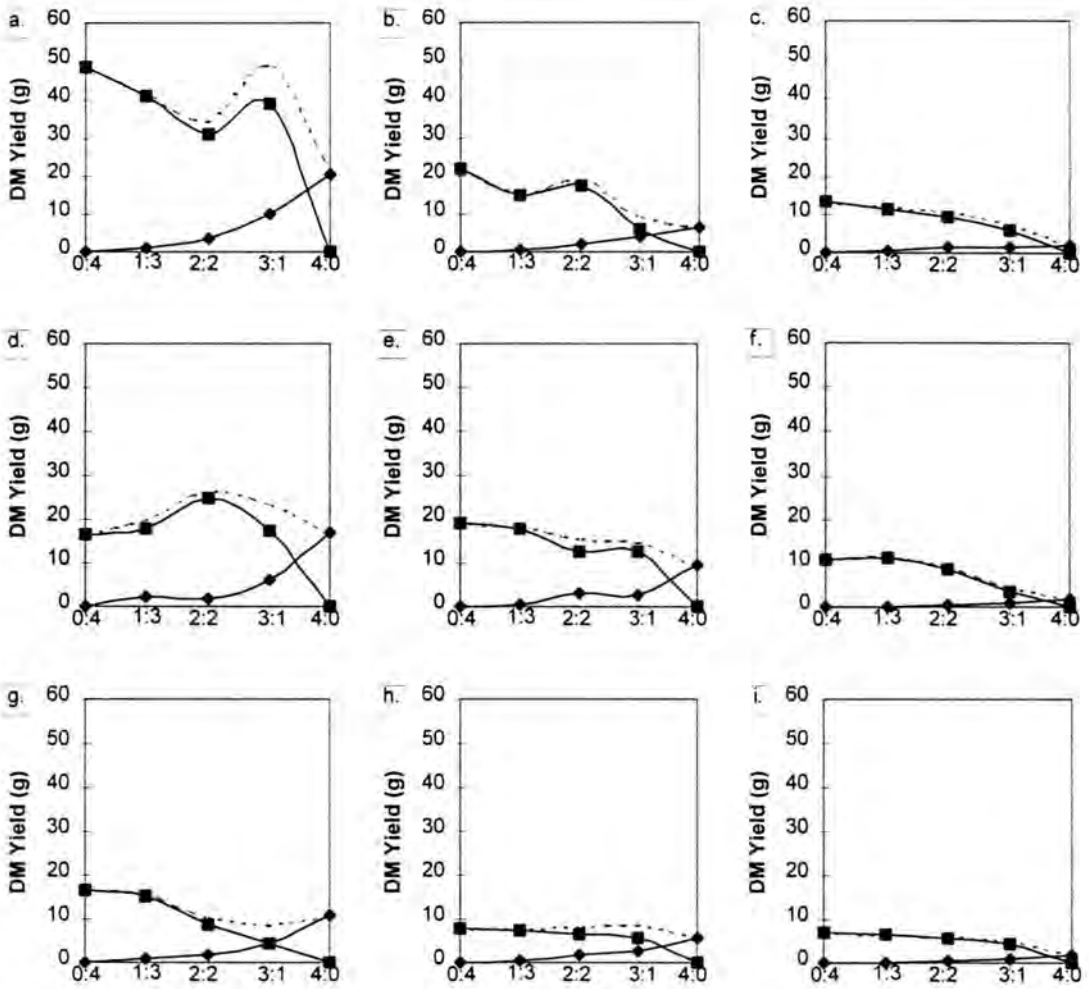

Figure 9. Replacement diagrams (total shoot mass) for *Acacia melanoxylon* and *Paraserianthes lophantha* grown in monoculture and in 1:1 mixtures under varying moisture/sunlight levels (Moisture/sunlight levels (%) are as follows: **a** = 100/100; **b** = 100/60; **c** = 100/20; **d** = 50/100; **e** = 50/60; **f** = 50/20; **g** = 20/100; **h** = 20/60; **i** = 20/20. ◆ = *A. melanoxylon* and ■ = *P. lophantha*. The dotted line indicates the total pot yield.).

for the mixture are very erratic, so that no clear trend can be distinguished (Table 4, Table 5).

Table 3. The relative yield totals (RYT) of species in mixtures under various moisture and light levels (a = *Acacia mearnsii*, l = *A. melanoxylon* and p = *Paraserianthes lophantha*).

Moisture	Light	Ratio (a:l, l:p, a:p)		
a/l		3:1	2:2	1:3
100	100	6.52	1.51	2.76
100	60	0.94	1.73	1.35
100	20	0.93	0.75	1.44
50	100	1.05	1.11	0.64
50	60	0.44	1.25	1.26
50	20	2.49	1.15	1.43
20	100	0.78	1.32	0.89
20	60	1.28	1.14	1.20
20	20	0.80	1.61	1.00
l/p				
100	100	1.30	0.82	0.90
100	60	0.86	1.07	0.75
100	20	1.18	1.49	1.03
50	100	1.41	1.61	1.20
50	60	0.90	1.00	0.97
50	20	0.86	1.20	1.18
20	100	0.66	0.67	0.97
20	60	1.21	1.13	1.03
20	20	1.06	0.97	0.99
a/p				
100	100	1.22	1.10	1.25
100	60	2.54	1.94	1.56
100	20	0.63	0.78	0.74
50	100	2.43	1.47	1.09
50	60	1.33	0.89	0.77
50	20	1.39	1.10	1.36
20	100	1.26	0.78	0.58
20	60	0.85	0.8	0.84
20	20	1.43	1.44	1.34

Table 4. The relative crowding coefficient (K) of species in mixtures under various moisture and light levels (a = *Acacia mearnsii*, l = *A. melanoxylon* and p = *Paraserianthes lophantha*).

Treatment		Ratio (a:l, l:p, a:p)					
Moisture	Light	3:1		2:2		1:3	
a/l		K_{al}	K_{la}	K_{al}	K_{la}	K_{al}	K_{la}
100	100	-0.06	4.52	2.34	16.92	-3.28	2.13
100	60	0.54	6.91	12.30	5.34	3.49	-1.57
100	20	1.44	3.58	1.49	1.73	3.45	-1.09
50	100	1.11	4.65	1.97	1.44	5.73	1.00
50	60	0.37	4.48	2.50	2.85	3.27	-1.85
50	20	-0.27	4.08	3.81	1.69	6.49	3.07
20	100	0.67	4.14	2.76	3.12	3.34	1.58
20	60	-5.73	3.87	1.47	5.56	4.00	7.03
20	20	0.68	4.23	-9.61	2.03	4.84	0.89
l/p		K_{lp}	K_{pl}	K_{lp}	K_{pl}	K_{lp}	K_{pl}
100	100	0.66	15.26	1.22	2.78	3.18	2.13
100	60	0.83	4.08	1.39	4.78	3.16	1.10
100	20	1.21	5.47	4.84	3.32	3.54	2.72
50	100	0.52	-62.02	1.11	-1.97	3.43	-4.18
50	60	0.44	8.65	1.50	2.96	3.13	4.70
50	20	0.71	4.49	1.62	5.37	3.43	-5.99
20	100	0.55	4.08	1.18	2.08	3.22	3.39
20	60	0.64	10.94	1.36	7.15	3.23	7.25
20	20	0.63	7.35	1.20	5.07	3.20	4.61
a/p		K_{ap}	K_{pa}	K_{ap}	K_{pa}	K_{ap}	K_{pa}
100	100	0.39	-34.67	1.06	-25.43	3.06	-1.39
100	60	-1.18	-11.85	2.06	-2.33	3.32	-0.72
100	20	0.56	3.87	1.28	2.26	3.32	0.94
50	100	-0.29	4.13	3.41	4.24	4.61	1.27
50	60	2.99	5.42	1.06	5.76	3.13	1.21
50	20	0.72	20.97	1.27	8.79	3.44	-1.44
20	100	1.49	5.82	1.06	3.61	3.52	0.59
20	60	0.35	16.08	1.03	4.47	3.05	1.82
20	20	0.58	-507.0	1.69	-31.69	4.06	-4.28

Table 5. The relative effect of inter-specific competition (R) of species in mixtures under various moisture and light levels (a = *Acacia mearnsii*, l = *A. melanoxylon* and p = *Paraserianthes lophantha*).

Treatments		Ratio (a:l, l:p, a:p)					
Moisture	Light	3:1		2:2		1:3	
a/l		R_{al}	R_{la}	R_{al}	R_{la}	R_{al}	R_{la}
100	100	0.66	-5.19	0.06	0.43	0.16	-0.91
100	60	0.43	0.62	0.19	0.08	-0.21	0.86
100	20	0.84	0.23	0.58	0.67	-0.31	0.87
50	100	0.64	0.3	0.70	0.51	0.33	0.52
50	60	0.67	0.89	0.35	0.40	-0.18	0.92
50	20	0.74	-1.23	0.59	0.26	0.11	0.46
20	100	0.72	0.5	0.32	0.36	0.21	0.90
20	60	0.77	-0.06	0.18	0.68	0.05	0.75
20	20	0.71	0.49	0.49	-0.10	0.38	0.62
l/p		R_{lp}	R_{pl}	R_{lp}	R_{pl}	R_{lp}	R_{pl}
100	100	0.20	0.50	0.36	0.82	0.16	0.94
100	60	0.74	0.40	0.21	0.72	0.30	0.95
100	20	0.55	0.28	0.30	0.21	0.12	0.85
50	100	-0.05	0.64	-0.51	0.90	-0.08	0.88
50	60	0.35	0.75	0.34	0.67	0.07	0.96
50	20	0.67	0.47	0.19	0.62	-0.06	0.87
20	100	0.74	0.61	0.48	0.85	0.10	0.93
20	60	0.27	0.52	0.14	0.73	0.05	0.93
20	20	0.41	0.53	0.20	0.83	0.07	0.94
a/p		R_{ap}	R_{pa}	R_{ap}	R_{pa}	R_{ap}	R_{pa}
100	100	-0.09	0.86	-0.04	0.94	-0.24	0.99
100	60	-0.25	-0.28	-0.43	0.49	-0.46	0.90
100	20	0.77	0.6	0.44	0.78	0.36	0.9
50	100	0.73	-1.16	0.24	0.29	0.26	0.65
50	60	0.55	0.11	0.17	0.94	0.27	0.96
50	20	0.14	0.46	0.11	0.79	-0.23	0.87
20	100	0.52	0.22	0.28	0.94	0.57	0.85
20	60	0.19	0.96	0.22	0.97	0.18	0.98
20	20	-0.01	0.58	-0.03	0.59	-0.08	0.74

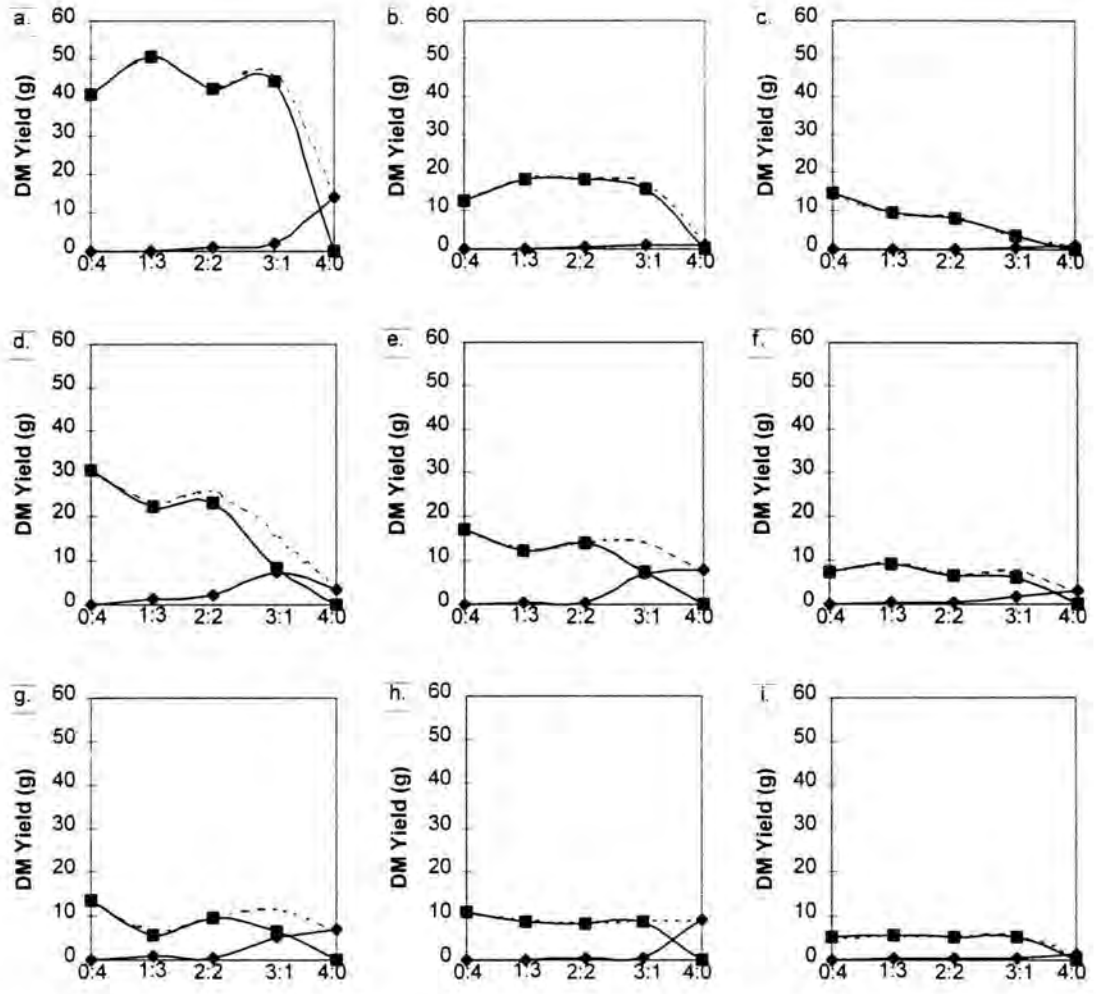

Figure 10. Replacement diagrams for *Acacia mearnsii* and *Paraserianthes lophantha* grown in monoculture and in 1:1 mixtures under varying moisture/sunlight levels (Moisture/sunlight levels (%) are as follows: **a** = 100/100; **b** = 100/60; **c** = 100/20; **d** = 50/100; **e** = 50/60; **f** = 50/20; **g** = 20/100; **h** = 20/60; **i** = 20/20. ◆ = *A. mearnsii* and ■ = *P. lophantha*. The dotted line indicates the total pot yield.).

A. melanoxylon/P. lophantha

The predominantly convex curves of *P. lophantha* compared to the concave curves of *A. melanoxylon* under almost all moisture/light treatment combinations indicate that *P. lophantha* is the better competitor (Figure 9a-i). This is confirmed by the R_{pl} and K_{pl} values which is generally bigger than the R_{lp} and K_{lp} values (Table 4, Table 5). The RYT-values show much smaller variation than in the above mentioned mixture and appear to average around unity, an indication that the two species largely share the same resources (Table 3). The growth of both species in monoculture are equally

reduced by shade stress but *P. lophantha* appears to be influenced more negatively than *A. melanoxylon* by moisture stress.

A. mearnsii/P. lophantha

Judging from the shape of the curves *P. lophantha* is also a better competitor than *A. mearnsii* under all moisture/sunlight conditions (Figure 10a-i). This is confirmed by the K_{pa} values which are generally bigger than the K_{ap} values (Table 4). The R-values are again erratic, but generally show that the relative effect of inter-specific competition of each species on the yield of the other one, increases with its frequency in the mixture (Table 5). The RYT-values again show no trend and vary greatly (Table 3). The variability of the RYT-values in both mixtures that involved *A. mearnsii* is probably due to the high level of variation that occurred in the growth of seedlings of *A. mearnsii* and therefore no conclusions about the demands of the species on the same resources could be made.

6.4 Discussion

P. lophantha is superior to the two *Acacia* species both in production of dry matter growth of shoots and roots under all nutrient concentrations. The higher production at the 25% nutrient level compared to the 50% and 75% nutrient levels could be explained through observations of McDowell and Moll (1981), who showed that *P. lophantha* performed better in a soil mixture without added nutrients than in a nutrient-enriched mixture. They concluded that *P. lophantha* would probably grow well in nutrient-poor sites. The excellent growth of *P. lophantha* under the 100% nutrient level is therefore contrary to the general trend and findings of McDowell and Moll (1981). It might be an indication that *P. lophantha* can grow equally well in a wide range of soils with differing degrees of nutrient richness.

Growth of *A. melanoxylon* and especially *A. mearnsii* is inhibited by the 100% nutrient solution. This is possibly an indication that both species are adapted to a natural habitat that is less fertile. This is logical because Australian soils are generally poor in nutrients (Beard 1983). The nutrient solution used in this experiment was developed for wheat production in glashouses. The results indicate that even at the 25% level, nutrient concentrations were probably sufficient for the species under

investigation, hence the lack of response to the higher levels. In similar experiments to be executed in the future, much lower nutrient levels should be applied.

In the second experiment *P. lophantha* again is superior to the other two species, both in respect of shoot length and -biomass. *P. lophantha* shows a positive response to increased moisture levels at the 20% sunlight level, while the two *Acacia* species show no response to increased moisture levels, indicating that light is the limiting factor. The ability to produce growth at the 20% sunlight level could be expected from *P. lophantha* due to the relatively large seed size. It has been shown that species with larger seeds produce seedlings that are more capable of growing under shaded conditions than species with smaller seeds (Ng 1978; Fenner 1983; Augspurger 1984; Seiwa & Kikuzawa 1991).

Shade level apparently do not influence the drought resistance or ability to grow under limiting moisture regimes in *P. lophantha* and *A. melanoxylon*. *A. melanoxylon* grows slightly taller under the 60% sunlight level at 20% moisture than at the 100% sunlight level (Figure 7b) but the trend is not reflected in the dry mass data (Figure 6b), indicating that it is a plastic response (Harper 1977). The same argument applies for *A. mearnsii* at the 20% sunlight/20% moisture levels. There is therefore no indication that seedlings are more drought tolerant when grown in deep shade as was found for *Pittosporum undulatum* (Gleadow & Rowan 1982). Growth responses in this study appear to be similar to those found by Kolb *et al.* (1990), that reductions in growth due to low levels of soil moisture for *Quercus rubra* and *Liriodendron tulipifera* were more pronounced in full sun than in shade. Similar trends were observed by Carter and Klinka (1992) for *Pseudotsuga menziesii, Tsuga heterophylla* and *Thuja plicata*.

An interesting trend reflected in all species (although to a lesser degree in *P. lophantha*), is the decrease in performance under 60% sunlight levels when moisture level is increased from 50% to 100% (Figure 6a,b,c and 7a,b,c). In the case of the two *Acacia* species, it could possibly be explained as follows: in full sunlight, the seedlings grow optimally and can utilize all the available water, while under 60% sunlight, growth of the plants - as well as evapotranspiration - is decreased, causing an oversupply of water in the pots which could be detrimental to growth. This

explanation could be relevant to *P. lophantha* too, but the positive response to higher water levels under the 20% sunlight regime is then contradictory.

The superior growth of *P. lophantha* to the two *Acacia* species can be explained by the larger seed size of *P. lophantha* (Chapter 2). Larger seeds usually result in larger seedlings which have an advantage over small seedlings at least in the first stages of life (Harper 1977). Reports of bigger seeds resulting in better initial seedling growth were made by Twamley (1967), McDowell and Moll (1981), Augspurger (1984), Gross (1984), Kolb and Steiner (1990), Kolb *et al.* (1990), Seiwa and Kikuzawa (1991) and Bi and Turvey (1994). However, Abbott (1984), found no correlation between seed weights and germination success, survival or increase in length.

Several studies indicated that seed size only benefits seedling growth in the initial growth phases, or else under adverse conditions (Augspurger 1984; Gross 1984; Kolb & Steiner 1990; Kolb *et al.* 1990). Therefore, under optimum growing conditions, large seeded species may lose their advantage in the late- or post-seedling stage. Observations on juvenile *A. longifolia* and *P. lophantha* plants co-occurring at Hermanus and Paarl Mountain, confirm this (Pieterse unpublished). *A. longifolia* produces seeds approximately similar in size to *A. mearnsii* and *A. melanoxylon*. About two years after fires at both locations, *A. longifolia* plants were not, as was expected, significantly smaller than *P. lophantha* plants. McDowell and Moll (1981), comparing the growth of three species including *A. longifolia* and *P. lophantha*, found that *P. lophantha* outgrows and outcompetes *A. longifolia* when seedlings were grown in monocultures and mixtures for twelve weeks. They did however notice that *A. longifolia* invested more in root growth than *P. lophantha* which would probably be to the advantage of *A. longifolia* in the future. The fact that *A. longifolia* grows as well as *P. lophantha* at Hermanus and at Paarl Mountain indicates that *A. longifolia* must gain on *P. lophantha* after the initial seedling growth stage. This confirms the theory that at some stage, the benefits of large seed size is lost and other factors dictate the growth rates of the species. Another factor slowing down the growth of *P. lophantha* could be the early transition to a reproductive stage, compared to the *Acacia* species. Taylor (1978) mentioned that *P. lophantha* is able to produce seeds within two years post establishment. More than 50% of the *P. lophantha* plants at Hermanus (18

months old) and Paarl Mountain (2 years old) produced flowers or seeds (Pieterse unpublished) - a confirmation of Taylor's observation. Waller (1988) stated that where plants continue to grow for several years, selection favours perennials that reduce or postpone reproduction, accumulating resources instead that allow them to overtop and dominate their neighbours (or subdue them in some way e.g. allelopathy). Instead of becoming reproductive, the *Acacia* species could allocate reserves into vegetative growth with the result that they might overtake *P. lophantha* eventually.

Kolb and Steiner (1990) stated that *Q. rubra* ecotypes with superior juvenile growth rate under competition-free conditions will also have superior growth rate under at least some conditions of shading and root competition. This theory could be extrapolated to the three species under investigation. The superior growth rate of *P. lophantha* under optimum conditions is not influenced by nutrient, moisture or light deficiencies when grown in monoculture. In addition, these factors did not greatly influence the superior growth rate of *P. lophantha* in mixtures (Figures 9 and 10). Therefore competing species did not cause a bigger water deficit that could retard growth, as was found by Withers (1979) and Perry *et al.* (1994). *P. lophantha* probably extracts water at a faster rate than the two *Acacia* species. Eissenstat and Caldwell (1988) have shown that the rate of water extraction influences competitive ability in *Agropyron desertorum* and *A. spicatum.*

In this study, although it is not the main objective to study competition, the replacement design is used for a preliminary investigation of the similarity of competing taxa and the competitive abilities of the species. The fact that the experiment is conducted at a single seedling density is a drawback and results obtained from the experiment should be interpreted with caution. These results give an indication of which species perform better than other species without addressing the reasons for it. A properly designed competition experiment involving varying seedling densities in monocultures too, is needed to confirm the results obtained in these experiments.

In the *A. melanoxylon/A. mearnsii* mixtures under different nutrient concentrations, no trends could be observed regarding the effect of nutrient concentration. Although not so clear from the replacement diagrams (Figure 3a-h), the R- and K-values

indicate that *A. mearnsii* has a bigger effect towards *A. melanoxylon* than *vice versa*. Under natural conditions *A. mearnsii* would probably compete even better than in the glasshouse (see comments in the results section). The large variation in seedling growth probably influences these results. Aarssen (1989) stated that genotypic variability in competitive ability is no greater between species than within species. The reason for the relatively weak growth and high mortality of *A. mearnsii* could possibly be due to mycorrhizal dependency of the species, because the experiments were conducted in sterilized sand. Both Tobiessen and Werner (1980) and Osonubi *et al.* (1991) have shown that mycorrhizal effects can greatly influence the survival of tree seedlings. In the second experiment it did appear as if *A. mearnsii* competed better at lower moisture levels than at higher levels - this is also reflected in better growth in the monocultures (Figures 6c and 7c). Sunlight levels do not seem to influence competitive ability of the two species. *P. lophantha* is a superior competitor to the two *Acacia* species as is shown by the convex curves produced under all tested conditions, at least for the 12 weeks that the experiment lasted.

These studies may give an indication of the outcome of competition in nature. Van Baalen (1982) cautioned that experiments carried out only with mixtures of plants of the same age, are likely to yield results of low predictive value. However, in the case of these three species, which are all adapted to germination after fire (Chapter 4) the chances are that in most competition situations, seedlings will be of uniform age.

The results of these studies confirm that seedlings derived from large seeds have an initial competitive advantage over small seeded species. They confirm results obtained by McDowell and Moll (1981) and Bi and Turvey (1994). However, the small seeded species can reverse the situation after the initial growth phases (Abbott 1984; Augspurger 1984; Gross 1984; Kolb *et al.* 1990). Therefore no conclusions over the long term outcome of competition between these species can be made. Any competitive studies regarding trees or perennial plants should therefore be conducted under natural conditions for at least two growing seasons. Any experiment like this one that only lasts for a few weeks is an exercise which cannot accurately predict the long term competitive outcome between species.

P. lophantha in particular is adapted to a wide range of environmental conditions and outperforms the other two species under all given conditions in the seedling stage. *A. mearnsii* and *A. melanoxylon* are both susceptible to heavy shading and *A. mearnsii* to wet conditions. According to Costermans (1981), in Australia *A. mearnsii* prefers drier, shallow soils. It is therefore hard to explain why *A. mearnsii* grows so well next to or even in river beds if seedlings do not tolerate wet conditions. Perhaps the nutrient status of the soil, soil type, drainage, mycorrhizal dependency etc. play a role in determining the survival of seedlings. Schönau and Schulze (1984) set an upper limit of mean annual precipitation of 1 200 mm for *A. mearnsii*, due to *inter alia* a higher incidence of the gummosis disease complex. They also set a lower altitudinal limit of 600 m to avoid the poor and diseased growth found in the warm and humid coastal belt of Kwazulu-Natal. Conditions under cover such as in glass houses could possibly be similar to those experienced in the coastal belt of Kwazulu-Natal.

The main conclusions drawn from these studies are therefore: 1) *P. lophantha* is a dangerous invader under most environmental conditions and can probably invade undisturbed indigenous vegetation. (Hypothesis i is therefore only partly corect, because *P. lophantha* is more shade resistant than *A. mearnsii*, but *A. melanoxylon* is not. The other two hypotheses are refuted by the results); 2) *A. mearnsii* and *A. melanoxylon* probably require disturbance for them to invade indigenous vegetation; 3) *A. mearnsii* would not invade excessively wet and fertile areas (which are scarce in the Fynbos Biome, except in riparian silt); 4) *P. lophantha* will outcompete the two *Acacia* species initially under most environmental conditions. However, data from other studies indicate that it may lose its competitive advantage in the post-seedling stage. The early seeding habit of *P. lophantha* might however give the species a competitive advantage in terms of population growth over the other two species, especially in areas subjected to frequent burns.

Part 2

Aspects of the control of woody invasive legumes

Chapter 7

Is burning of a standing population a viable control method? Effects of a wildfire on an *Acacia mearnsii* population.

7.1 Introduction

Acacia mearnsii De Wild. is an important invasive species in the Fynbos Biome (Macdonald & Jarman 1985), Eastern Cape (Henderson 1992), Natal (Henderson 1989) and Transvaal (Henderson & Musil 1986). *A. mearnsii* is a fire-adapted pioneer plant in Australia, its country of origin (Sherry 1971). Fire is therefore beneficial for the germination of the hardseeded seeds in the soil; a feature that can be used to control the large soil stored seed bank in the soil (Hendry & Van Staden 1982). It has been proposed that fire be used as a tool to control the large soil stored seed banks under Australian acacias (Milton & Hall 1981; Pieterse & Cairns 1986; Holmes *et al.* 1987; Holmes 1989b). Arguments against the use of fire in alien invader control programmes have been presented recently (Breytenbach 1989; Macdonald 1991a; Macdonald & Wissel 1992). Fire has also been implicated as the main disturbance factor that can create "invasion windows" allowing alien invasive plants to establish in natural fynbos (Richardson & Cowling 1992; Richardson *et al.* 1992). There appears to be a good reason to study interactions between alien invasive plants and fire.

In January 1989 a wildfire swept through an area in the Hottentots Holland mountains destroying many hectares of fynbos as well as a large area infested with *A. mearnsii* next to the Theewaterskloof Dam. This presented an opportunity to investigate the effects of fire on a standing population of *A. mearnsii*. Information thus obtained could shed some light on the interaction between population growth and fire, as well as the feasibility of using fire as a control tool against *A. mearnsii*.

7.2 Material and methods

Study area

The study area was situated between the northern shore of the Theewaterskloof dam and the Hottentots Holland mountains (19°10'E;34°02'S).

Effect of fire on the population

Fifteen 10 m X 10 m quadrats were located in areas where the density of *A. mearnsii* plants varied before the fire in March 1989. In each plot 20 soil cores were randomly taken to a depth of 200 mm by means of a soil auger with diameter of 75 mm and depth of 200 mm. The soil samples were put into plastic bags, taken to the laboratory and wet-sieved by means of a Retsch automatic sieving machine with a mesh size of 1.2 mm. Seeds were extracted by hand after the sieving process. Seeds were inspected and classified as hard or charred. Hard seeds were considered viable as it was established previously that *ca.* 99% of hard seeds from soil samples taken after a burn were viable (Pieterse 1986).

In each plot the number of *A. mearnsii* seedlings that established after the fire were recorded. Additionally, 20 seedlings were excavated around the perimeter of each plot. The germination depth of each seedling was determined by measuring the distance from the flange to the soil surface. This distance gives an accurate indication of the germination depth (Pieterse 1986).

In each plot the number of recognisable *A. mearnsii* plants from the charred remains were recorded to obtain an indication of the pre-fire plant density and of the stem diameter at 20 mm above the soil surface. These data were used to calculate the plant density and total basal stem area for each plot. The plants were arranged into four arbitrarily chosen size classes, according to stem diameters, as follows: class 1 (seedlings) = 0-25 mm stem diameter; class 2 (juveniles) = 26-50 mm stem diameter; class 3 (young trees) = 51-100 mm stem diameter and class 4 (adult trees) = > 100 mm stem diameter. The plants were arranged into three classes depending on their condition *viz.* alive (green, living material present in the canopy), coppicing (all material in the canopy burnt brown and dead but coppicing from the base of the stem) and dead (no green canopy material and no coppicing). The percentage of dead,

coppicing or live trees in each size class (over all plots) was calculated. The pre-fire density (dead, coppicing and living stems) was compared to the post-fire density (live and coppicing stems as well as seedlings). Regression analyses were performed on some of the parameters to establish whether there was any relationship between them (Table 1).

Population expansion by seedling spread from parent populations

Several clumps of trees that formed well defined borders in the burnt area were selected randomly in an area of about 15 ha. Twenty transects orientated perpendicular to the edge of the infestations, were laid out. From the edge of the *A. mearnsii* infestations, the transects extended into natural vegetation where no sign of pre-fire *A. mearnsii* plants could be detected. The natural vegetation consisted mainly of grasses and sedges with some forbs interspersed between them. Transects were one meter wide and 20 meters long, divided into one meter segments resulting in 20 X 1 m^2 quadrats extending from the edge of the infestation. All seedlings in each quadrat were recorded in March 1989. The transects were extended by an additional 80 m line along which any *A. mearnsii* seedlings within 1m from the line, were recorded. The 20 m transects were again inspected in December 1989 and in December 1990. The mean number of seedlings at different distances from the edge of the infestations was calculated as well as the percentage mortality of the seedlings during periods between counts.

Seedling density and size at varying distances from parent populations

In December 1989, five of the transects described in the previous section, in which the most seedlings occurred, were selected. Similar transects were located 500 mm from the first transects for vegetation production determinations. These transects however only extended for 10 m away from the edge of the infestation into the natural vegetation. All five transects were situated parallel to each other within a radius of 50 m. The 10 m transects were again subdivided into ten 1 m^2 quadrats. In each quadrat, all living material was cut at ground level and sorted into *A. mearnsii*, grasses/sedges and forbs. The material were placed in a glasshouse and left to become air dry. After 2-3 months, the material was placed into a drying chamber at 40°C and dried to a

constant weight. The total dry mass of *A. mearnsii* seedlings, the odd *A. longifolia* seedlings, grasses/sedges and forbs were determined for each quadrat. Additionally, the stem diameter at ground level, height and dry mass of each individual *A. mearnsii* and *A. longifolia* plant were determined. Regression analyses as indicated in Table 2 were performed to investigate possible relationships between parameters.

In December 1990, another five transects similar to and 500 mm apart from the 1989 transects, were laid out and plant material was harvested as before. This time however, due to a lack of time and labour, only the stem diameters of the *A. mearnsii* seedlings were determined. Stem diameters were arranged into 10 mm interval classes and for each transect, 20 seedlings from each stem diameter class were selected to determine the dry mass and height. The relationship between stem diameter and dry mass was determined by means of regression analyses and these equations were used to estimate the dry mass of the *A. mearnsii* seedlings in the transects. The number of seedlings as well as their dry mass were compared to the same parameters as were used in 1989. The dry mass of the grasses/sedges and forbs were also determined.

7.3 Results

Effect of fire on the population

The pre-fire density of established plants ranges from five stems plot^{-1} (100 m^2 plots) to 543 stems plot^{-1} and the total basal area from 1 058 mm^2 to 145 217 mm^2 (Table 1). Small seedlings present before the fire would have been destroyed by the fire without leaving a trace so that pre-fire density excludes such seedlings. After the fire the seedling numbers range from 60 seedlings plot^{-1} to 1 998 seedlings plot^{-1} and the number of viable seeds from 12 m^{-2} to 5 314 m^{-2} (Table 1). Unfortunately the wet sieving process resulted in many of the charred seeds disintegrating and the seed numbers given in Table 1 therefore only refer to viable seeds that survived the fire. The effect of the fire on the viability of the seeds is therefore unknown. The mean seedling germination depth ranges from 14.7 mm to 27.2 mm. The deepest observed germination depth is 62 mm. The pre- and post- fire densities are shown in Figure 1. The mean factor by which populations have increased, is 21.8. Figure 2 indicates that

the lowest number of trees killed are adults with the mortality percentage increasing with decreasing size. The juvenile trees (Size class 2) have by far the largest percentage of coppicing plants. The only significant regression coefficients are obtained for density *vs.* total basal stem area (r^2=0.78), which is quite logical, and density *vs.* seed bank (r^2 =0.71) (Table 2).

Table 1. The number of stems and total basal area plot^{-1} before the burn, as well as number of seeds, seedlings and mean germination depth in fifteen plots after the fire.

Plot	Density (stems plot^{-1})	Total basal stem area	Number of seedlings plot $^{-1}$	Germination depth (mm)	Number of seeds m^{-2}
1	7	1058.15	60	22.9±10.19	65.0±114.3
2	31	2326.65	84	16.80±8.9	116.9±257.31
3	52	1830.38	544	19.50±9.2	493.8±524.9
4	54	2821.85	566	23.40±14.2	285.9±415.8
5	75	2637.14	1998	23.20±11.8	649.7±465.2
6	5	1452.18	263	24.5±10.2	13.0±57.2
7	543	5544.98	234	21.9±13.1	649.7±675.7
8	25	2056.8	498	18.10±7.5	558.7±545.7
9	12	1568.91	756	17.50±8.6	129.9±215.7
10	161	3699.86	663	27.20±9.9	1299.4±771.8
11	33	2819.76	411	22.35±11.6	363.2±371.6
12	76	2622.61	308	25.30±9.3	337.8±395.0
13	43	3355.49	1194	23.20±8.8	65.5±114.3
14	13	2391.49	260	21.00±13.2	91.0±210.5
15	28	1876.83	1542	14.70±6.6	5314.3±2195.9

Table 2 Regression coefficients of selected parameters determined in fifteen 10 m X 10 m quadrats laid out in *A. mearnsii* infestations after a wildfire.

Parameter	DF	Equation	r^2
Density *vs.* total basal stem area (TBSA)	14	y = -350.13+804.89(log(x))	0.78
TBSA *vs.* number of seedlings	14	y = x /(-0.002*x +12.85)	0.3
TBSA *vs.* number of seed	14	y = x /(-0.009*x +42.77)	0.18
Seed number *vs.* number of seedling	14	y = 478.61 + 0.211*x	0.26
Density *vs.* number of seedling	14	y = x /(0.002*x +0.034)	0.18
Density *vs.* number of seed	14	y = x /(-0.004*x +0.289)	0.71

Population expansion by seedling spread from parent populations

The mean distance from the furthest seedling to the edge of the infestation is *ca.* 20 m, with a maximum of 29 m. The seedling numbers m^{-2} decrease with increasing distance from the edge of the infestation in March 1989 (Figure 3). The same trend, although less conspicuous, appears in December 1989 and December 1990 (Figure 3). If the total number of seedlings in all transects are considered, seedling mortality from March 1989 to December 1989 is 47.2%, from December 1989 to December 1990 is 80.4%, resulting in a total seedling mortality

Table 3. Regression coefficients of several parameters harvested in five transects extending from the edge of an *Acacia mearnsii* infestation in 1989.

Parameter	DF	Equation	r^2
Distance from edge *vs.* total seedling weight	45	Y = 276.56 * EXP (-0.54*X)	0.54
Distance from edge *vs.* mean seedling weight	45	Y = 1/(2.37 + 1.31*X)	0.21
Grass & forb mass *vs.* total seedling mass	45	Y = 736.63 - 107.2 * LOG (X)	0.45
Grass mass *vs.* total seedling mass	45	Y = 141.4 * EXP (-0.004*X)	0.42
Grass + Forb + total seedling mass *vs.* mean seedling mass	45	Y = 1/6.78 + 0.003*X)	0.15
Total grass mass *vs.* mean seedling mass	45	Y = 0.47*EXP (-0.002*X)	0.24
Total seedling mass *vs.* mean seedling mass	45	Y = 0.04 + 0.004*X	0.74
Seedling number *vs.* mean seedling weight	45	Y = X /(6.81*X + 94.6)	0.37
Grass & forb mass *vs.* mean seedling mass	45	Y = 2.92 + -0.41*LOG (X)	0.22
Forb mass *vs.* mean seedling mass	45	Y = 1/(10.5 + -0.02*X)	0.09
Seedling number *vs.* total seedling mass	45	Y = X /(-0.27*X + 29.06)	0.96

of 89.6% from March 1989 to December 1990. Although a much larger number of seedlings die nearer to the edge of the infestation, the percentage mortality of seedlings over the whole range of the transects stays relatively stable at *ca.* 90%.

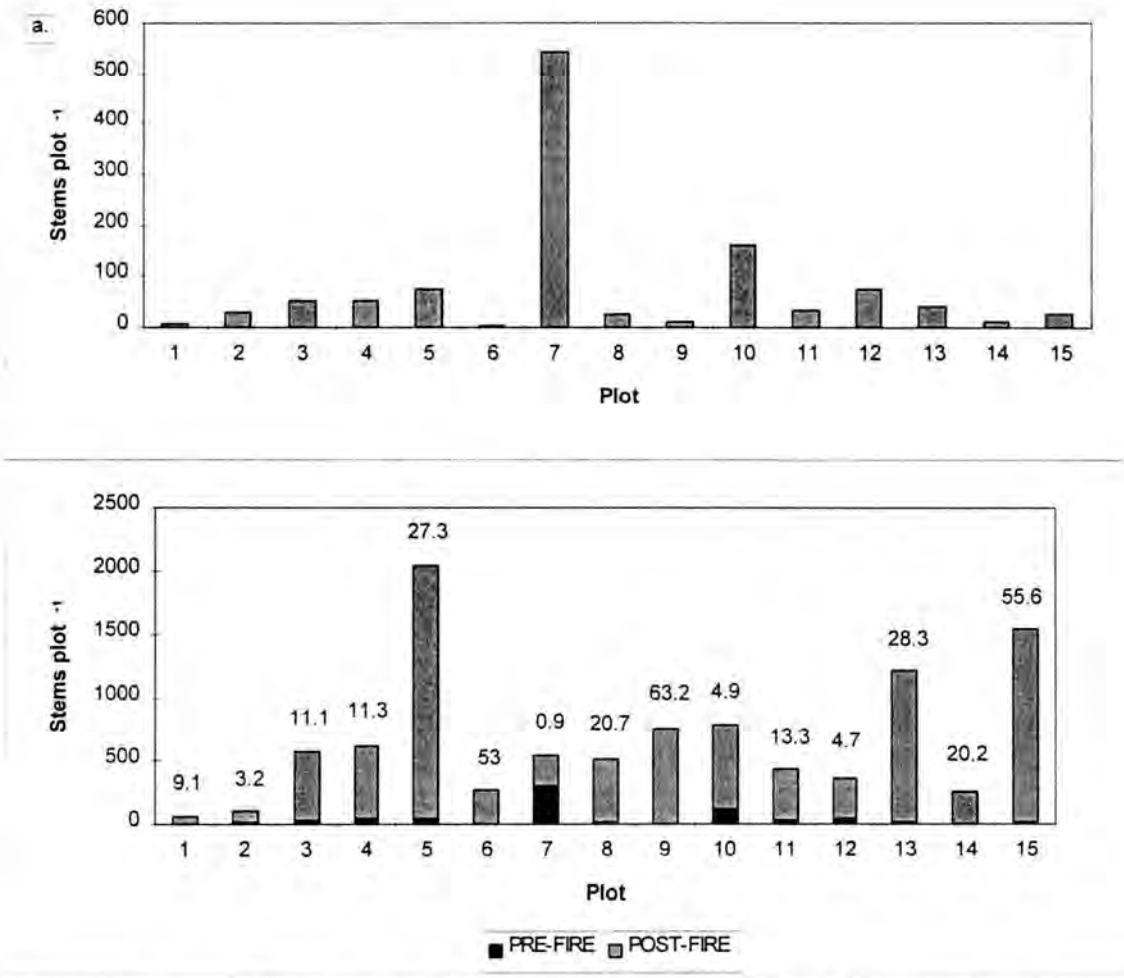

Figure 1 **a.** The stem density plot^{-1} of *A. mearnsii* before the burn as calculated from the remaining stems in 15 plots; **b.** The pre- and post-fire densities of *A. mearnsii* in 15 plots. The factor by which the population has grown, is given at the top of the bars.

Seedling density and size at varying distances from parent populations

Figure 4a indicates that dry matter production of grasses/sedges and forbs is decreased near the edge of the infestation. This could be a result of the high *A. mearnsii* seedling number near the edge (Figure 4b) or possibly through the shading effect of surviving plants. Figure 4c indicates that the biggest plants occur near the edge of the infestation. Again it raises the question whether the bigger size of the seedlings near the infestation is due to the lower dry mass of the grasses and forbs or due to a better microclimate. However, no significant regression between grass dry mass and total seedling mass or mean seedling mass could be established (Table 3). The only

significant regression coefficients occur between seedling number and total seedling mass (which is logical) and between total seedling mass and mean seedling mass. This is again logical - the more seedlings, the smaller each individual.

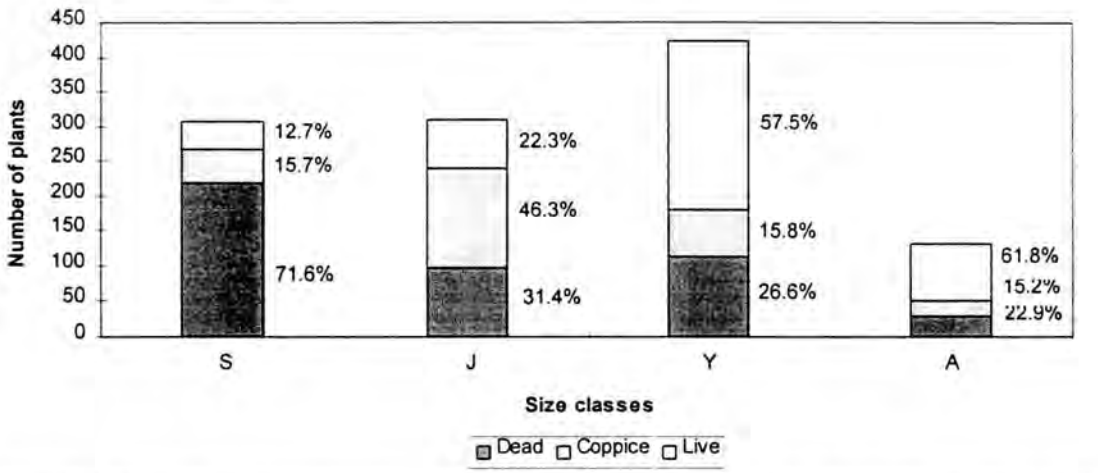

Figure 2 The numbers of dead, coppicing and live *Acacia mearnsii* plants in the different size classes after the wildfire (S = 0-25 mm stem diameter, J = 25-50 mm, Y = 50-100 mm and A = >100 mm).

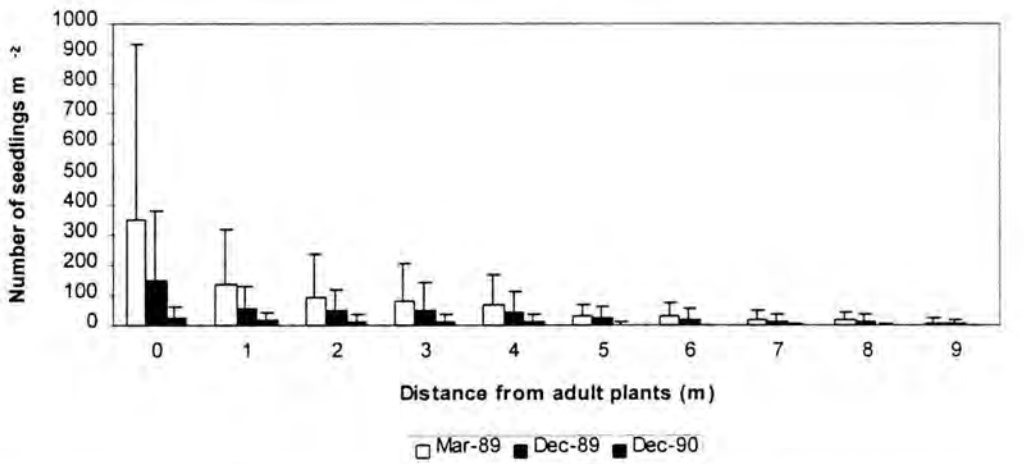

Figure 3 The number of seedlings in a $1m^2$ quadrat at increasing distances from the edge of an *Acacia mearnsii* infestation at three different sampling dates after the wildfire. (Vertical bars indicate standard deviation).

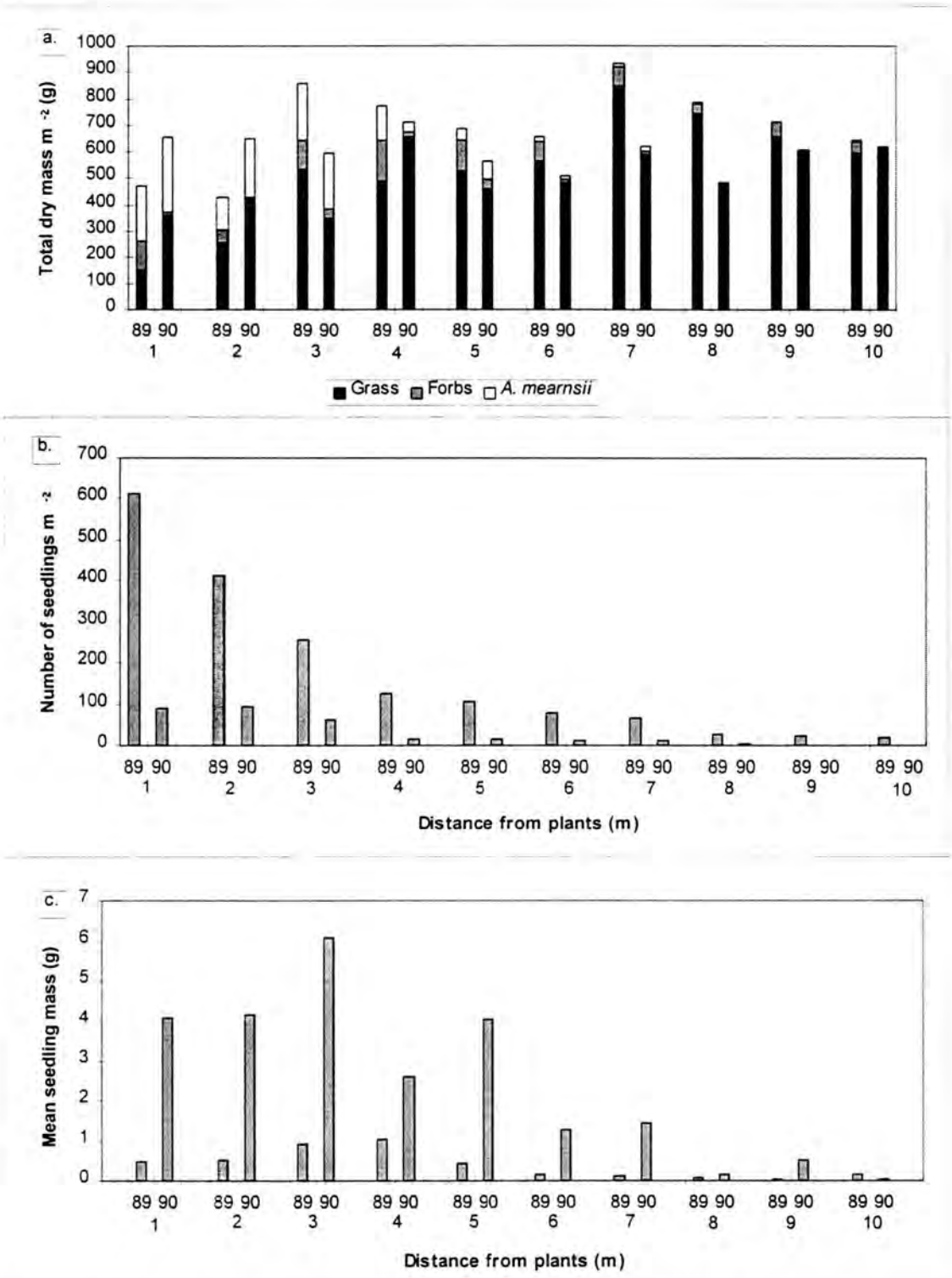

Figure 4 **a.** The total dry mass of grasses, forbs and *Acacia mearnsii* seedlings at two harvest times in $1m^2$ quadrats 1-10 m from the edge of an infestation; **b.** The number of *A. mearnsii* seedlings at two harvest times in $1m^2$ quadrats 1-10 m from the edge of an infestation; **c.** The mean seedling mass of *A. mearnsii* at two harvest times in $1m^2$ quadrats 1-10 m from the edge of an infestation.

7.4 Discussion

The use of fire to reduce the soil seed reserves of alien invasive acacias has been investigated by several researchers (Milton & Hall 1981; Hendry & Van Staden 1982; Pieterse 1986; Pieterse & Cairns 1986; Holmes *et al.* 1987; Pieterse & Cairns 1987; Holmes 1989b). Vermeulen (1989) and Campbell (1993) recommended that, under certain conditions, fire be used as part of integrated control programmes. Most of these recommendations involved fire after the existing plant population was felled. Pieterse & Cairns (1987) and Holmes (1989b) suggested that it could be more economical to burn standing populations of alien plants without felling them first. The results from this study indicate that, as far as the above-ground population is concerned, burning of a standing population of *A. mearnsii* is not a feasible proposition. Regeneration results in the post-fire population being unacceptably high. Although it can be argued that the seedlings will be exposed to natural mortality, as is observed in the seedling populations in the transects, the plants that survived the fire are of the greatest concern. The large percentage of resprouting plants will make follow-up control difficult. Resprouting, multi-stemmed trees are harder to control (Macdonald 1991) and result in an escalation of the cost of control (Macdonald & Wissel 1992). Apart from the increase in population size and the difficulty to control resprouting plants, the presence of charred remains can hamper the control of the seedlings, especially if the original stand was dense.

The data lost when the charred seeds from the seed bank could not be retrieved, prevents the determination of the effect of the fire on the seed bank. The data on the depth of germination of the seedlings may, however, provide indications about the intensity of the fire as well as the effect of the fire on the seed bank. In this study, the mean germination depth of the seedlings in the different plots varied between 14.7 and 27.2 mm. In a study where an *A. longifolia* population was felled and burnt, Pieterse and Cairns (1986) found that the most seeds were ready to germinate at a depth of 20 mm. Holmes (1989b) found that most *A. cyclops* seedlings germinated at 20-30 mm depths after a fell and burn treatment, but where the plants were burned standing, most seedlings germinated at 40-50 mm depths. Pieterse (1986) found that the mean depth of *A. longifolia* seedlings that established after wildfire swept through a standing

population, was 29.8 mm, while 80% of the seed bank was destroyed. In Australia, mean germination depths of *Acacia* species following fires were given as >12.5 mm by Cunningham and Cremer (1965), 24.4 mm by Auld (1986*b*) and 10-30 mm by Bradstock and Auld (1995). The mean depth of germination in this study was slightly smaller than in the standing burn cases reported by Pieterse (1986) and Holmes (1989b). Extrapolating from their data, it seems likely that at least 80% of the seed bank would have been killed by the fire in this study.

In terms of control of the seed bank, therefore, it appears as if felling or not felling before burning is not critical. Holmes (1989b) speculated that felling beforehand concentrated the fuel in the lower layers thereby slowing the rate of spread and the rate of heat release resulting in a lower intensity fire. Holmes's data suggest some correlation between fire intensity and amount of heat transfer below ground. In contrast, Breytenbach (1989) found that *Hakea* spp. that were slashed and burned gave a higher intensity fire than when burned standing. Burning standing *Hakea* spp. resulted in a lower intensity fire than burning fynbos vegetation. In a fire simulation study, Van Wilgen and Richardson (1985) showed that the burning of standing *A. saligna* thickets should result in a lower intensity fire than the burning of fynbos. Breytenbach (1989). however, warned that the use of fire models have limited application from a biological point of view, because heat delivered at the soil surface is more important than the total heat released into the atmosphere. A low intensity, back burning fire, therefore has a more serious effect on plants and soil than a high intensity, fast moving fire. This hypothesis is supported by Hodgkinson and Oxley (1990) who found that litter fuel burns slowly and promotes germination of fire adapted shrubs more than fire fuels that burn fast. Bradstock and Auld (1995) could also detect no relationship between the Byram fire intensity and either maximum temperature change or duration of heating in the soil between 60°C and 120°C. Temperatures exceeding 60°C in the upper 30 mm of soil were only reached under some slow moving fires. There appears to be some uncertainty about the relationship between fire intensity and the effect of the fire on organisms below soil level.

These studies show that the edge of an infestation can be expanded by about 20 m due to establishment of seedlings after a fire. This is a slow expansion due to short

distance dispersal, probably by ants (Chapter 2) and possibly by gravity and/or wind. The more rapid long distance expansion due to water transport probably does not rely on fire to aid establishment of seedlings. This is in correspondence with the findings of Lepart and Debussche (1991) who stated that the mean wind dispersal distance of *Pinus halepense* was 24 m. They also distinguished between water dispersal of *Fraxinus ornus* seeds which was more rapid (*ca.* 1 km year^{-1} downstream) than wind dispersal (*ca.* 20 m year^{-1} upstream). The fire has two effects on the *A. mearnsii* population *viz.* increased post-fire density of the population relative to the pre-fire density and expansion of the edges of the population. It is therefore imperative to attempt to control the seedlings following a wildfire. This will prevent the infestation from expanding and in the case of a less dense stand interspersed with indigenous vegetation, from smothering the indigenous vegetation. A fire will also provide the opportunity for chemical control of resprouting trees, although it will be more expensive than spraying unburned shrubs (Macdonald & Wissel 1992).

The fact that the same percentage of seedlings (not the same number) over the whole transect survives after 18 months, indicates that the seedlings experienced the same competition intensity from the indigenous vegetation further away from the edge of the infestation than from its siblings nearer to the edge of the infestation. The fire provided the 'gap' in the surrounding indigenous vegetation for the seedlings to establish. The theory that fire is a key factor providing a 'gap' or 'invasion window' for the invasion of indigenous vegetation by invasive aliens, has been suggested by Lepart and Debussche (1991), Specht and Clifford (1991), Richardson and Cowling (1992) and Richardson *et al.* (1992). Trabaud (1991), in contrast, stated that invasion of indigenous vegetation by invasive plants after a fire in the Mediterranean region, California chaparral and the sclerophyll forests of Australia is only temporary and the fire-adapted indigenous plants generally outcompete the invasives after a few years. Why this does not occur in fynbos, is probably due to the lack of trees in the fynbos vegetation (Moll *et al.* 1980) or the lack of rapid regeneration of a dense ground cover. The indigenous vegetation of the fynbos, consisting mainly of low shrubs, probably cannot outcompete the invasive trees such as *A. mearnsii*. It is, however, interesting that the dense grass/restio vegetation which developed within three months did not suppress the *A. mearnsii* seedlings to a greater extent, because Stubbings and

Schönau (1983) advised that grassland should be plowed to a depth of 100 mm to kill grasses to ensure successful establishment of *A. mearnsii*.

This study verified the hypothesis that fire is beneficial for establishment of invasive plants in pure indigenous vegetation and it also benefits the above ground population growth of the plants, although the seed bank was decreased. From a control point of view, it appears as if a standing burn of *A. mearnsii* plants, although it might reduce the seed bank, will result in more problems during the follow-up operations than is gained economically during the initial clearing phase. The standing burning of *A. mearnsii* is therefore not recommended. It is however clear, that where a wildfire has swept through a standing population, it is imperative to control the resulting seedlings to prevent the population from expanding.

Chapter 8

The utilization of woody legume invaders as mulches: a preliminary study

8.1 Introduction

Various methods other than biological have been proposed to control invasive species (Macdonald *et al.* 1985). However the most effective and economic control measure appears to be an integrated control programme. Using this method, mechanical, chemical and biological control measures are integrated in the most efficient way possible for the particular conditions (Kluge *et al.* 1986). The successful establishment of these programmes can be extremely expensive. Integrated control programmes for *Acacia saligna* in fynbos areas could, depending on plant size, plant density and the control methods used, cost up to R25 000.00 ha^{-1} (Macdonald & Wissel 1992). More economical alternative control methods are therefore required.

In a changing South Africa, the number of small-scale and subsistence farmers will probably increase considerably. In the south-western Cape, with its hot, dry summers, these farmers will have to be established near perennial streams and rivers to provide for their irrigation requirements. These are the kind of habitats preferred by the three woody legumes considered in this paper (Macdonald & Jarman 1984).

Small-scale farmers and more specifically, subsistence farmers, will probably not have the financial means to fertilize the soil as recommended by agriculturists. They will therefore have to opt for sustainable low-input management systems. One way of achieving this goal would be to make use of green manure provided by woody legumes (Kang *et al.* 1990).

In alley cropping systems where arable crops are grown between wide rows of trees (Lal 1991), branches are pruned regularly to provide leaf mulch for the interplanted crop. The quality of the mulch does, however, influence the release of nitrogen from the mulch (Kachaka *et al.* 1993; Handayanto *et al.* 1994). Factors such as a high polyphenol content, high lignin content and a low *in vitro* dry matter digestibility (IVDMD) of mulch material

may result in a slow decomposition and therefore a slow nutrient release rate (Gutteridge 1992). In contrast, species with a high IVDMD and low levels of polyphenols, lignin etc. will probably have a faster nutrient release rate (Gutteridge 1992). The ideal mulch may therefore be a mixture of a long lasting type of mulch and a rapid release type (Gutteridge 1992). In this study, *Chamaecytisus palmensis* (tagasaste) was included as a rapid release type, because of its relatively high IVDMD (Table 1).

The objective of this study, therefore, is to establish whether i) an economical way of controlling invasive woody legumes and ii) providing small scale farmers with suitable material for mulching, can be achieved simultaneously by utilising the leaf material of woody invaders growing naturally near the fields of small scale farmers.

8.2 Material and methods

For all experiments, young leaves and twigs were clipped from *A. mearnsii*, *A. melanoxylon* and *P. lophantha* trees growing in the vicinity of Stellenbosch (33°56'S;18°52'E). The *C. palmensis* clippings were obtained from trees growing at Camphill Village (33°40'S;18°25'E). Samples of the dried clippings were analysed for dry matter content, ash, crude protein, fibre, IVDMD, acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin (Table 1).

Table 1 The chemical composition of clippings obtained from the various species used in the experiments.

	Acacia mearnsii	*Acacia melanoxylon*	*Paraserianthes lophantha*	*Chamaecytisus palmensis* *
ASH	4.29	4.55	5.32	N/A
CRUDE PROTEIN	15.22	13.86	15.69	18.0
FIBRE	25.45	30.97	15.55	N/A
IVDMD	41.10	44.60	52.27	71.0
ADF	28.78	37.70	17.35	27.0
NDF	34.82	45.63	22.70	41.0
LIGNIN	25.31	33.65	14.66	7.3

* Data from Borens & Poppi (1990) and Lambert *et al.* (1989).

The analyses were performed by the Animal Nutrition Laboratory at Elsenburg Agricultural Development Institute using standard methods (AOAC 1984). The soil used in the pots was collected on Welgevallen Experimental Farm in Stellenbosch and is a grey, loamy sand with a pH of 5.1 (KCL), an organic carbon content of 0.56%, a total nitrogen content of 0,095% (Kjehldahl method) and an available phosphorus content of 40 mg kg^{-1} (Bray 1).

Experiment 1

The first experiment was designed to assess the nitrogen fertilizer equivalence of clippings from the four legumes mentioned above. The experiment was performed in a glasshouse at the University of Stellenbosch between 28 June and 19 November 1993. Day/night temperatures of *ca.* 20/10°C occurred for most of the time but temperatures rose to 25-30°C on hot days towards the end of the experiment. Clipped leaves and twigs of up to 5 mm diameter were ovendried to a constant weight at 60°C.

Table 2 Chemical and plant fertilizer treatments applied to pot-grown wheat plants.

1	0 kg NH_4NO_3 ha^{-1}	
2	25 "	
3	50 "	
4	100 "	
5	150 "	
6	*Acacia mearnsii*	2.5 t dry matter ha^{-1}
7		5.0 "
8	*Acacia melanoxylon*	2.5 "
9		5.0 "
10	*Paraserianthes lophantha*	2.5 "
11		5.0 "
12	*Chamaecytisus palmensis*	2.5 "
13		5.0 "
14	*A. mearnsii* and *C. palmensis* (1:1)	2.5 "
15		5.0 "

The dried clippings of each of the four species and one treatment where clippings of *A. mearnsii* and *C. palmensis* were mixed in a 50:50 ratio was applied at two rates (Table 2) to the surface of 20 cm diameter plastic pots each containing 3.5 kg of soil. The effect of these 10 treatments was calibrated using five fertilizer nitrogen treatments in which NH_4NO_3 was applied to separate pots at five rates (Table 2). KH_2PO_4 was applied in solution form to all pots to provide the equivalent of 65 kg K ha^{-1} and 30 kg P ha^{-1}. *Triticum aestivum* L. (CV SST44) kernels were sown into the pots and thinned to two healthy wheat seedlings pot^{-1} two weeks after planting.

Pots were watered every second day to field capacity. Drainage water was collected in plastic trays under each pot and returned, to prevent nitrogen leaching from the pots. Plants in pots were harvested at 8, 11, 14, 17 and 20 weeks after the planting date. (The experiment was designed to have a pot for each of these dates.) Plants were harvested by cutting at the soil surface, after which they were oven dried for 96 hr at 60°C, weighed and ground in a mill to pass through a 1 mm sieve. The nitrogen content of the seedlings was determined by processing on an automatic analyser (Technicon Infra-alyzer 400). Nitrogen production, the product of nitrogen content and dry mass of the plants, was calculated for each pot. Because the nitrogen production results closely resembled the dry weight results, only dry matter (DM) yield will be considered in the analysis of the data.

The experimental design was a randomised complete block design (RCBD), with two blocks. There was a total of $5 \times 15 = 75$ pots (experimental units) per block. The treatment design was such that the pots were assigned at random to all combinations of five harvest times (ages at which the two healthy wheat plants growing in a pot were harvested) and 15 allocations of fertilizer, whether in the form of plant material or chemicals. In five of the 15 treatments, nitrogen was applied at levels equivalent to 0, 25, 50, 100 and 150 kg N ha^{-1}, to allow the estimation of a curve showing the response of wheat to nitrogen fertilizer. In the remaining 10 of the 15 treatments, plant material was used as fertilizer. Four species and a mixture involving two species were applied at one of two levels. The 15 fertilizer treatments, labelled 1 to 15, are shown in Table 2.

Treatment means were calculated using the LSMEANS statement of PROC GLM and comparisons between treatments were made using the CONTRAST statement of PROC GLM (SAS Institute Inc. 1985).

Experiment 2

The second experiment was designed to determine if there was any interaction between varying levels of clippings and nitrogenous fertilizer applied to *Zea mays* (CV Jubilee) plants growing in pots. The experiment was conducted between 24 December 1993 and 23 March 1994 in a glasshouse at temperatures ranging between 35°C and 15°C. Two maize plants were established in plastic pots, without drainage holes and containing 3.5 kg of soil and the plants were thinned to one plant per pot after two weeks. Oven-dried clippings of *A. mearnsii*, *A. melanoxylon* and *P. lophantha* trees were applied separately to pot surfaces at rates equivalent to 0, 2.8 and 5.6 t dry matter ha^{-1}. Nitrogenous fertilizer (NH_4NO_3 dissolved in water) was applied at rates equivalent to 0, 50 and 100 kg N ha^{-1}. KH_2PO_4 was added as in Experiment 1. The pots were brought to field capacity, weighed, and then kept at field capacity by adding the required amount of tap water every second day. The plants were harvested at five intervals *viz.* 4, 6, 8, 10 and 12 weeks after planting as described in Experiment 1. At each harvest time, the dry mass of the plants were determined. At harvest times 1, 2 and 3 the weights of the pots were recorded before being brought to capacity to determine the amount of water lost since the previous watering.

The experimental design was a completely randomised design (CRD) with two pots (experimental units) for every treatment. The treatment design was a 5×3×3×3 factorial (i.e. a total of (5×3×3×3)×2 = 270 pots, were used). The factors were Age (4, 6, 8, 10 and 12 weeks), Fertilizer (applied at rates equivalent to 0, 50 and 100 kg N ha^{-1}), Species (plant material from *A. mearnsii*, *A. melanoxylon* and *P. lophantha* was applied) and Mulching Rate (application was at rates equivalent to 0, 2.8 and 5.6 t dry matter ha^{-1}).

Results

Judging from the IVDMD figures and the fibre and lignin content, *C. palmensis* and *P. lophantha* should decompose faster than *A. mearnsii* which in turn should decompose faster than *A. melanoxylon* (Table 1).

Table 3 ANOVA table for Experiment 1 where the log(dry matter) of pot grown wheat in reaction to five levels (0, 25, 50, 100 and 150 kg N ha^{-1}) of nitrogenous fertilizer and two levels (2.5 and 5 t ha^{-1}) of five mulch treatments (*Acacia mearnsii*, *A. melanoxylon*, *Paraserianthes lophantha* and *Chamaecytisus palmensis* and a mixture of *A. mearnsii* and *C. palmensis*) was determined at five harvest times (ages). (The CONTRAST statement was used to test for significant differences between mulch levels of species).

Source of variation	df	MS	F	SL
Blocks	1	1.2443		
Age = A	4	22.7959	217.87	0.0001
Fertilization = F	14	1.9664	18.79	0.0001
Level of *A. mearnsii*	1	0.1520	1.45	0.2319
Level of *A. melanoxylon*	1	0.0002	0.00	0.9620
Level of *P. lophantha*	1	1.4584	13.94	0.0004
Level of *C. palmensis*	1	0.5493	5.25	0.0248
Level of mixture	1	1.0110	9.66	0.0027
A×F	56	0.1120	1.07	0.3892
Error	74	0.1046		

The analysis of variance shown in Table 3 is a standard analysis of a RCBD, viewing the 75 treatments in a block as forming a 5×15 factorial, with factors age and fertilization. No interaction occurs between age and fertilizer, but the main components age as well as fertilizer had significant effects on the dry-matter production of the wheat plants.

The contrasts in Table 3 suggest that the amount of dry matter produced does not vary with varying amounts of *A. mearnsii* and *A. melanoxylon*, but that this amount does vary in the case of *P. lophantha* and *C. palmensis*, and a mixture containing *C. palmensis*. A possible explanation for this is that the latter two species have softer leaves, with less lignin (Table 1) and probably with less tannin, implying that nitrogen is released sooner by these species than by the *Acacia* species. The observed means (averaged over the five ages) are plotted in Figure 1.

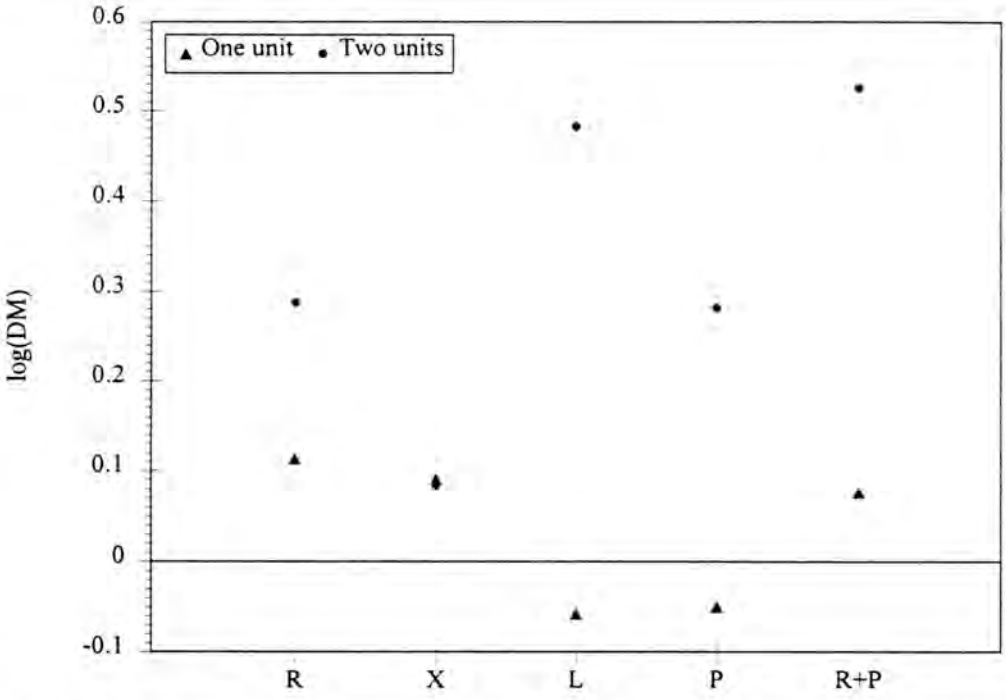

Figure 1 The relationship between the logarithm of the amount of dry material of pot-grown wheat plants and the level (one or two units) of plant material. (R = *Acacia mearnsii*, X = *Acacia melanoxylon*, L = *Paraserianthes lophantha*, P = *Chamaecytisus palmensis*).

Table 4 ANOVA table for the fifth harvest (age = 20 weeks) of Experiment 1 where the dry matter yield of pot grown wheat in reaction to five levels (0, 25, 50, 100 and 150 kg N ha^{-1}) of nitrogenous fertilizer and two levels (2.5 and 5 t ha^{-1}) of five mulch treatments (*Acacia mearnsii*, *A. melanoxylon*, *Paraserianthes lophantha* and *Chamaecytisus palmensis* and a mixture of *A. mearnsii* and *C. palmensis*) was determined.

Source of variation	df	MS	F ratio	SL
Blocks	1	1.1714	1.35	
Nitrogen,	1	123.9941	97.81	0.0001
A. mearnsii,	1	3.4374	2.71	0.1219
A. melanoxylon,	1	2.4550	1.94	0.1858
P. lophantha,	1	2.9155	2.30	0.1517
C. palmensis,	1	0.0605	0.05	0.8303
Mixture,	1	2.7473	2.17	0.1631
Error	14	1.2677		

In an attempt to obtain the final effect of the treatments, the dry matter measured at the fifth (last) age was analysed (untransformed). The results of this analysis are shown in Table 4. Nitrogen application significantly influenced dry matter production. No mulch treatment has any significant effect on dry matter production.

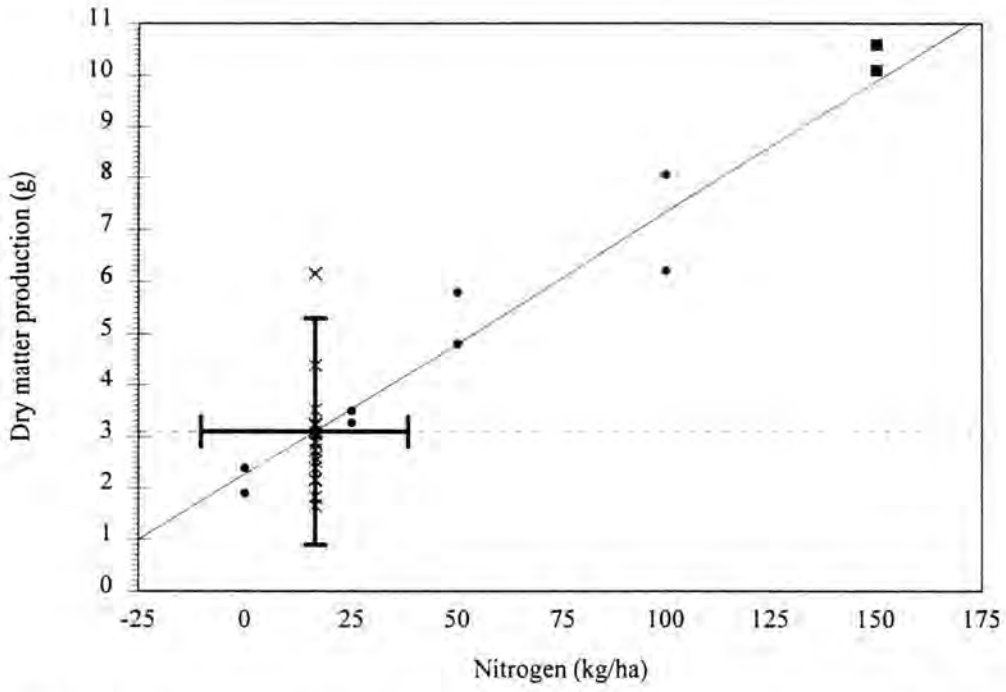

Figure 2 Using the relationship between dry matter production of pot-grown wheat and fertilization to calibrate the species for their effective amount of nitrogen.

The 30 observations for this sub-experiment are plotted in Figure 2. A straight line was fitted to the observations of the first four levels of nitrogen applied (the dots). This line is used in a calibration of the species. The curvature suggested in the previous analysis is not apparent in Figure 2, but it was felt safer to discard the observations of the fifth level (the squares). The curvature noted in the joint analysis of all ages was least apparent in the data for this age.

The 20 observations (two per species level, or mixture level) for the fertilization's with plant material are plotted as crosses. The mean of these 20 observations is 3.09, indicated by the horizontal dotted line. The vertical dotted line allows one to read off the point of intersection with the fitted regression line; it is located at 16.4 kg N ha^{-1}. The vertical interval includes 1.96 standard deviations either side of this mean, showing that only one of

the observations offers evidence in contradiction of the true species means being all the same. The 20 observations have therefore all been plotted at 16.4, the amount of nitrogen giving a response of 3.09. This calibrated amount of effective nitrogen in the plant material is however not significantly different from zero, as shown by the calibration (horizontal) interval. Thus, this analysis suggests that the sources of plant material do not differ and that they contribute no nitrogen.

The results of this experiment are therefore inconclusive. The full analysis suggests that *P. lophantha, C. palmensis* and the *A. mearnsii/C. palmensis* mixture contribute nitrogen, particularly when applied in large quantities (the second level) but the analysis at the fifth age is based on too few observations to reflect these differences significantly.

Experiment 2

The ANOVA in Table 5 indicates that mulch level of different species cause significant differences in dry matter production of maize plants. Table 5 also indicates a two-way interaction between age at harvesting and level of fertilizer that was applied. The species means are shown in Table 6.

Mulching with *A. melanoxylon* produces significantly more dry matter than mulching with any of the other two species does. Mulching with *P. lophantha*, in turn, produces more dry matter than mulching with *A. mearnsii* (Table 6).

The amount of plant material applied (mulching rate) has the mean responses shown in Table 7. Significant differences occur between all levels of mulch applied, but there is a much larger response from 0 t ha^{-1} to 2.8 t ha^{-1} than from 2.8 t $ha^{-1}$to 5.6 t ha^{-1}. Figure 3 illustrates the interaction between age at harvesting and fertilizer level. At early ages there is little or no difference in the response to the various fertilizer increases, but the difference becomes larger at later ages.

Table 5 ANOVA table of Experiment 2 where the log(dry matter) yield of maize plants in pots were recorded at five time intervals after planting. Mulch from three legume species (*Acacia mearnsii*, *A. melanoxylon* and *Paraserianthes lophantha*) was applied at three levels (0, 2.5 and 5 t ha^{-1}) together with nitrogen fertilizer applied at three levels (0, 50 and 100 kg N ha^{-1}) in a 5×3×3×3 factorial experiment.

Source of variation	df	MS	F ratio	SL
Age = A	4	8.5631	1723.40	0.0001
Fertilizer = F	2	2.0539	413.36	0.0001
A×F	8	0.1156	23.27	0.0001
Species = S	2	0.0374	7.53	0.0008
A×S	8	0.0045	0.91	0.5081
F×S	4	0.0022	0.44	0.7804
A×F×S	16	0.0058	1.16	0.3052
Mulching rate = R	2	0.1545	31.10	0.0001
A×R	8	0.0079	1.60	0.1300
F×R	4	0.0096	1.94	0.1082
A×F×R	16	0.0026	0.52	0.9305
S×R	4	0.0084	1.70	0.1535
A×S×R	16	0.0069	1.40	0.1526
F×S×R	8	0.0051	1.04	0.4111
A×F×S×R	32	0.0053	1.07	0.3794
Error	135	0.0049		

Table 6 Species means of log(dry matter) of pot-grown maize plants (averaged over all ages). The standard error of a mean in the table is 0.00743. (Means followed by a different letter differs significantly at the 5% level)

Species	Mean
A. mearnsii	3.113c
A. melanoxylon	3.154a
P. lophantha	3.135b

Table 7 The effect of mulching rate (averaged over species) on the log(dry matter) of pot-grown maize plants. The standard error of a mean in the table is 0.00743. (Means followed by a different letter differs significantly at the 5% level)

Mulching rate	Mean
0.00 t dry matter ha^{-1}	3.087c
2.8 "	3.148b
5.6 "	3.167a

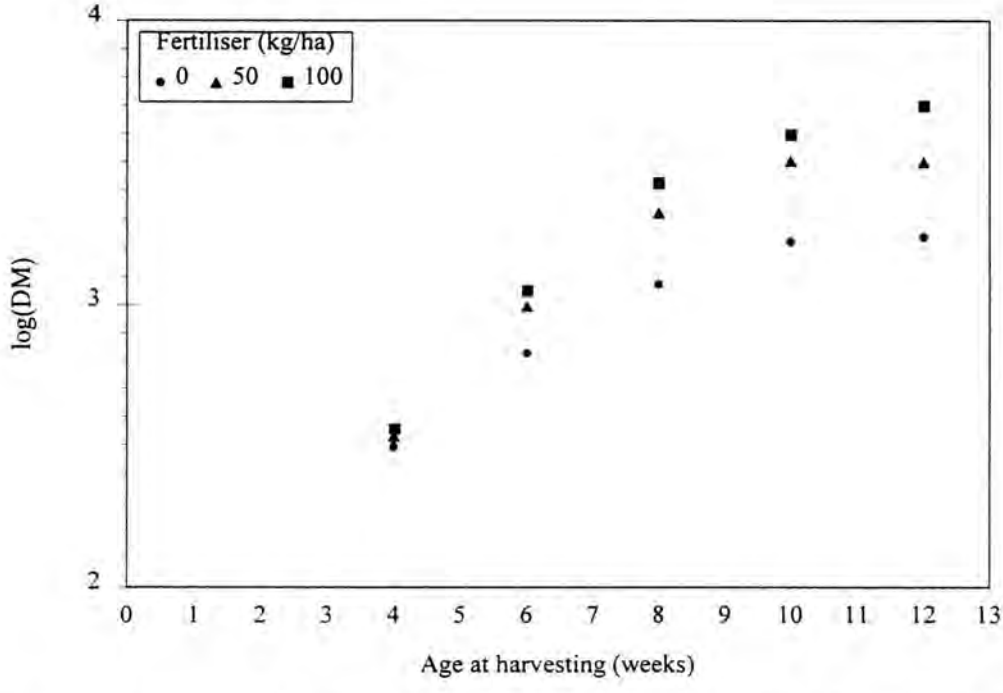

Figure 3 The interaction between the amount of fertilizer applied and the age at harvesting of pot-grown maize plants.

Unfortunately, because of an unintentional failure to record full information, a valid statistical analysis could not be performed on the data on the loss of water in the pots at the three harvesting times. However, there are tantalising patterns in the data which must be pointed out. A 3×3×3 table of mean water losses (harvesting at 4, 6 or 8 weeks × three species × mulching at rates equivalent to 0, 2.8 or 5.6 t ha^{-1}) reveals a consistent reduction

in loss when mulching at 2.8 t ha^{-1} when compared to no mulching (Figure 4). However, doubling the rate of mulching does not seem to have a consistent advantage.

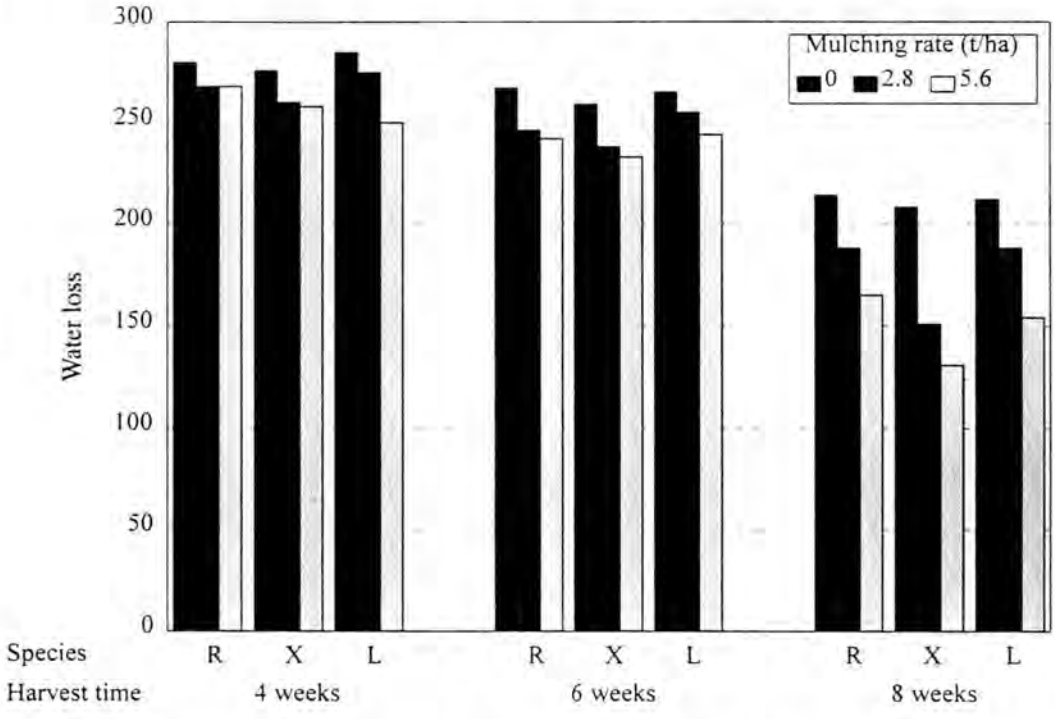

Figure 4 The loss of water from pots at three harvest times, three mulching rates and for three species (R = *Acacia mearnsii*, X = *A. melanoxylon*, L = *Paraserianthes lophantha*).

8.3 Discussion

Experiment 1

If the average dry matter production over all five harvests is considered, only *P. lophantha*, *C. palmensis* and the *A. mearnsii*/*C. palmensis* mixture showed significantly higher dry matter production when the amount of mulch applied was doubled from 2.5 t to 5 t ha^{-1}. It is interesting to note that in all three instances a species with low lignin content is involved, either alone or in a mixture. In the *Acacia* species, however, higher mulch levels did not appear to contribute to a higher dry mass production. The relatively poor performance of the higher level of *A. melanoxylon* mulch treatment is hard to explain. Possible reasons are that the *A. melanoxylon* leaves could have an inhibitory effect on wheat, or the denser covering of the pot surface by *A. melanoxylon* phyllodes could have altered the temperature and moisture regimes in the pot to the detriment of the wheat plants. *A. mearnsii*, on the other hand had a higher dry matter production at the lower level of mulching compared to

the lower levels of the other treatments. The results are therefore somewhat confusing, but the positive effects of the higher levels of mulch in the "softer" species should be investigated further.

The inferred nitrogen content of the mulches, indicated by the horizontal line in Figure 2, are generally between 10 and 30% of the actual N content determined analytically. These results are similar to those recorded by Gutteridge (1992) for some indigenous Australian legumes. The slow release of nitrogen from the mulch could be due to the chemical composition of the leaves. Factors such as lignin content, polyphenol content, lignin/N ratio, (lignin+polyphenol)/N ratio, C/N ratio, the protein binding capacity of the polyphenols and the soluble/insoluble ratio of polyphenols could play a role in the delay of nitrogen release from mulches (Kachaka *et al.* 1993; Handayanto *et al.* 1994). Gutteridge's (1992) recommendation that a rapid release type such as *Sesbania* (in this case *C. palmensis* was chosen) be combined with a long lasting type, was not supported. The *C. palmensis* treatment did not show the expected early response, indicating that nitrogen was still not released satisfactorily. It should however be kept in mind that Experiment 1 was conducted at low temperatures (10/20°C night/day) and microbial action at these temperatures was probably slow. If mulch decomposition rate increased towards the end of the experiment, when temperatures were higher, it would not have been evident from the dry matter production, because growth at that stage had already slowed down.

Another factor could be that the nitrogen released from the mulch, went into the organic soil N pool, from where it is mineralised over a long period into inorganic N that is available for uptake by plants. Only 10% of the ^{15}N labelled nitrogen applied as mulch in a particular year was found in the crops of that specific year (Haggar 1994). Therefore, a long term study is needed to follow the path of freshly released nitrogen from mulch.

Experiment 2

The rationale behind this experiment was the fact that nitrogen in the soil is first incorporated by the microbial agents involved in the decomposition of the leaf litter (Fitter & Hay 1987). This happens when the C:N ratio is about 20:1 to 30:1. Constantinides and Fownes (1993) have demonstrated that mulch containing a high percentage of twigs immobilises N in the soil during the degradation process, probably due to a higher lignin content and a higher C:N ratio of the twigs. It was postulated that the nitrogen addition

would neutralise the combined effect of nitrogen incorporation by microbial agents and the immobilisation of nitrogen by woody materials.

The lower level of fertilizer applied (50 kg ha^{-1}) did improve the effect of the mulch markedly but the trend was not reflected in the higher level of fertilizer that was added. It could not be a matter of immobilisation of the nitrogen by the higher mulch level, for the trend was not observed at the lower fertilizer level. It could however be the result of the threshold production for a pot being reached where other elements or root size became limiting. The fact that the mulch covered the soil in a layer about 10 mm thick could also result in only the material in direct contact with the soil being decomposed at a relatively fast rate, while that not in contact with the soil did not break down at an optimal rate. The fact that this experiment was conducted in summer, at high temperatures, also resulted in the top layer of the mulch becoming desiccated between times of watering, which would also have influenced decomposition rates of the top layer in the higher mulch level treatments.

In this experiment a significant difference between species was detected when the data for all five harvest times were taken into consideration. Surprisingly, *A. melanoxylon* mulch appeared to give the best results. In Experiment 1, however, *A. melanoxylon* mulch caused a poor response in dry matter production. A possible explanation could be that the better moisture retention capabilities of the phyllodes of this species, which affected the growth of wheat negatively in Experiment 1, could have been beneficial for maize growth in the hot conditions prevailing in the glasshouse during the duration of the experiment. Although the pots were brought to field capacity every second day, the soil under *A. melanoxylon* could have remained near or at field capacity during Experiment 1, which was conducted under cool conditions in winter. It has been shown that a mulch of wheat straw can inhibit wheat growth under cold, wet conditions (Ellis 1979), and the same could have happened under the *A. melanoxylon* mulch. On the other hand, in Experiment 2, which was conducted in summer under high temperatures, the pots tended to become quite dry towards the second day, especially towards the end of the experiment, when the maize plants rapidly depleted the moisture in the pots. Under such conditions the better water conservation properties of the *A. melanoxylon* mulch would have benefited the growth of the maize plants.

Although a relatively small percentage of the nitrogen in the leaf mulch was made available to the plants, the results of these preliminary trials indicate that the use of these mulches might be a viable option. The addition of mulch generally caused an increase in dry matter production, although not significantly in Experiment 1, in both trials. It was not determined whether this was due to nitrogen release, moisture conservation or possibly due to cooler soil temperature effects, and this requires further study. No serious negative effects due to allelopathic factors were observed, except possibly in the case of *A. melanoxylon* on wheat. It seems likely therefore, that the addition of mulches under field conditions will be beneficial to crop growth due to suppression of weeds and moisture conservation. Rippin *et al.* (1994) obtained increased maize yields with addition of mulches from *Erythrina poeppigiana* and *Gliricidia sepium* as opposed to unmulched control treatments. Higher yields were ascribed to weed suppression. Kiepe and Rao (1994) found that mulch alone conserved 42% of the rainfall and 83% of the soil lost from a pure crop without any conservation practices. Tilander (1993), working under semi-arid conditions, found that soil in plots receiving a high dosage of mulch, remained moist 2-3 days longer than other unmulched plots.

Alley cropping is not recommended in areas with low moisture and low soil fertility due to competition between crops and trees (Ong 1994). However, in this case, mulch material is to be harvested from invasive trees growing nearby. Large trees could be felled for firewood and the leaves and twigs used as a mulch. Younger trees could be pruned on a regular basis for a sustainable source of mulch material. By removing the leaves and twigs from those areas where the trees grow, a flow of fertility to the fields of the farmers should occur. The minerals under the trees will however be depleted (Haggar 1994), and the area where the trees grow would slowly be degraded to a less fertile environment. Such a degradation is preferable from two points of view; 1) lower soil fertility will decrease growth of the invasive plants, while 2) it will improve growth conditions for the indigenous fynbos vegetation which is adapted to infertile soils (Milton 1980b). The lower growth rates also improves the quality of the firewood. Harvesting should, however, not be undertaken when ripe seeds are present on the plants.

A system such as the one discussed above, will also be beneficial to integrated weed control, especially if trees could be harvested in Spring, when the invasive plant species

under discussion are flowering. By removing all or most of the flowers, seed set will be prevented or severely decreased, which will be beneficial to an integrated control programme. One of the major problems related to the implementation of such a system is the co-operation of the small-scale farmers themselves. It will be difficult to convince farmers to embark on such a labour-intensive operation if no definite economical reward can be offered.

The south-western Cape is faced with a severe water shortage which may become crucial before turn of the century (Little 1995). The use of mulch to conserve soil moisture and thereby reduce the amount of irrigation water needed is therefore of strategic importance. On the other hand, removal or reduction of the leaf biomass of the plant invaders growing on stream banks will help to conserve water, as these plants can reduce stream flow compared to indigenous plants growing in the riparian zones (Versfeld & van Wilgen 1986; Le Maitre *et al.* 1995). From a water conservation point of view, it is therefore important that this system prove to be successful.

These preliminary trials show enough merit for continuing the research under field conditions, where the rewards in terms of weed competition reduction and moisture conservation could be decisive. The long term effect of mulch application on soil properties in these areas also requires investigation.

Chapter 9

General discussion

9.1 Regenerating capacity and dispersal

An investigation into the dispersal characteristics of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha* (Chapter 2) reveals that, in South Africa, *A. mearnsii* is adapted to ant dispersal and *A. melanoxylon* to bird dispersal, as is the case in Australia (O' Dowd & Gill 1986), but that *P. lophantha* is not a myrmecochore, as was previously believed (Pemberton & Irving 1990). The hypothesis stating that seed dispersal characteristics in South Africa are similar to those in Australia, is accepted. *A. mearnsii* and *P. lophantha* maintain a reserve of dormant seeds in the soil (Boucher 1978, Taylor 1978). These two species therefore display the vital attribute 'S' (for long lived seed store) (Noble & Slatyer 1980). These plants are unlikely to become locally extinct, due to seed reserves in the soil, but cannot invade new habitats without the assistance of people. However, water dispersal may provide the means for limited long distance dispersal to enable the plants to invade new habitats (Dean *et al.* 1986). *A. melanoxylon* also maintains a reserve of dormant seeds in the soil but the seed is dispersed by birds (Moll 1978). The species therefore displays the vital attribute 'D' (for seed dispersal) which enables it to invade new habitats (Noble & Slatyer 1980). *A. mearnsii* and *A. melanoxylon* resprout from damaged trunks and the latter also suckers from the roots (Vermeulen 1989). *P. lophantha* does not resprout (Taylor 1978).

The relative invasive ability of the three species can thus be rated in terms of regenerating capacity and dispersal adaptations as follows:

$$\mathbf{I_{RD} = L + A + W + S + C + R}$$

where

I_{RD} = invasive ability in terms of regenerating capacity and dispersal adaptation

L = long distance dispersal by birds;

A = short distance dispersal by ants;

W = long distance dispersal by water;

S = long lived seed bank;

C = coppicing from stems;

R = suckering from roots.

The species are rated out of a possible score of 5. The more inducive to invasibility the characteristic is, the higher the score. The ratings are estimated by making use of knowledge available from the literature, results of this study, unpublished information available as well as some extrapolation from other studies. The species are rated as follows:

I_{RD} *A. mearnsii* $= 0 + 5 + 5 + 5 + 3 + 0$

$= 18$

I_{RD} *A. melanoxylon* $= 5 + 1 + 1 + 5 + 5 + 5$

$= 22$

I_{RD} *P. lophantha* $= 0 + 0 + 5 + 5 + 0 + 0$

$= 10$

In terms of regenerating capacity and dispersal ability, therefore, the species are ranked as follows: 1) *A. melanoxylon*, 2) *A. mearnsii* and 3) *P. lophantha*.

9.2 Seed and testa characteristics

Seed size is often an indication of the ecological habitat that a species occurs in (Salisbury 1942, Baker 1972, Fenner 1983). The seed sizes of the three species indicate that *P. lophantha*, having the largest seed and therefore the most available energy reserves, should be able to establish under conditions of deeper shade and from deeper germination depths than the other two species (Chapter 2). The other two species have seeds of roughly the same size, but *A. mearnsii* has a much higher oil content, indicating a higher energy content. *A. melanoxylon*, on the other hand, has a higher content of more of the important nutrients, including P, K, Ca and crude protein. These facts indicate that *A. mearnsii* would establish from deeper germination depths than *A. melanoxylon*, but that the latter would probably establish and grow better in nutrient-poor conditions than the other two species.

A. melanoxylon differs from the other two species in terms of the relative thickness of the palisade layer and mesophyl layer, indicating that the species does not lose its dormancy quickly (Morrison *et al.* 1992). The hypothesis stating that cell wall anatomy is not related to mode of dispersal, was refuted and an anatomical study of the seed of a larger number of ant- and bird-dispersed species is needed. All three species produce a high percentage of viable, dormant seeds.

The invasive ability of the species with regard to seed and seed testa characteristics is ranked according to the following formula:

$$\mathbf{I_{ST} = S + E + N + L + V + D}$$

where

I_{ST} = invasive ability in relation to seed and seed testa characteristics;

S = seed size;

E = energy content, *i.e.* oil and protein content and seed size;

N = nutrient composition, *i.e.* ability of seeds to establish under adverse conditions;

L = longevity, as indicated by the testa composition;

V = viability;

D = dormancy

The species are rated as follows (out of 5):

I_{ST} *A. mearnsii* = 3 + 5 + 2 + 2 + 5 + 5

= 22

I_{ST} *A. melanoxylon* = 3 + 2 + 5 + 4 + 5 + 5

= 24

I_{ST} *P. lophantha* = 5 + 2 + 3 + 2 + 5 + 5

= 22

Therefore, the species are ranked as follows: 1) *A. melanoxylon* with *A. mearnsii* and *P. lophantha* in joint second place.

9.3 Response of seed to dormancy breaking treatments

Seed of the three species all react to heat treatments (Chapter 4), but *A. mearnsii* is more resistant to heat treatments. *A. melanoxylon* is the most sensitive to heat treatments. It responds faster to heat treatments of increasing intensity but is also killed more rapidly. It can be argued that *A. melanoxylon* is well adapted to fire because of its fast reaction to less severe heat treatments, but being bird dispersed, most seeds in the soil seed bank will probably be on top or just below the soil surface, where it could easily be destroyed by fires. It is therefore concluded that it is not as well adapted to fire as the other two species. It does, however, react more quickly to sulphuric acid treatments, an indication that it is more adapted to bird dispersal than the other two species. *A. mearnsii*, although the diaspore is not adapted to bird dispersal, appears to be almost as sensitive to sulphuric acid treatments as *A. melanoxylon* is. *P. lophantha* shows no response to acid treatments at all. Therefore, the hypothesis that acid scarification will be a better dormancy-breaking mechanism for bird-dispersed species and that heat treatments will be better for ant-dispersed species, is accepted.

It was said earlier that *A. melanoxylon* is not adapted to fire because it gets killed quicker than the other two species by heat treatments. It might therefore sound contradictory to state that *A. melanoxylon* is adapted to bird dispersal because it reacts more rapidly and is killed more readily by acid scarification than the other two species. However, the gastric acid of birds are less concentrated than sulphuric acid, and seeds will not be killed as rapidly as is the case when exposed to sulphuric acid. There appears to be some relation between the composition of the seed testa and the sensitivity to acid, but not with sensitivity to heat.

Wet heat breaks the dormancy of seeds more quickly than dry heat, but dry heat fatally injures seeds more rapidly than wet heat. In practice, therefore, it should be decided whether the objective of burning is to kill most of the seeds in the seed bank or just to stimulate them to germinate. In the case of *A. melanoxylon* and *P. lophantha*, whose seeds should be relatively shallow in the seed bank, and which are sensitive to fire, it might be better to try to kill as many seeds as possible. Therefore a burn in the season when the soil is relatively dry and the soil is heated deeply, will be preferable. Burning in late November or December, even January, might destroy most of the seeds and seedlings that do germinate, will probably die from a lack of water during the rest of the dry season. *A. mearnsii*, with relatively heat resistant seeds that are buried by ants, will probably require a burn aimed at stimulating most seeds to germinate. These seedlings can then be controlled chemically or manually or left to be killed by drought. Such a burn should therefore be applied earlier, when enough soil moisture is still available. The effect of such fire on the surrounding indigenous vegetation should, however, always be borne in mind.

The invasive ability of the species in terms of resistance to dormancy breaking treatments, is estimated as follows:

$$\mathbf{I_{DB} = F + A}$$

where

I_{DB} = invasive abilities in terms of resistance to dormancy breaking treatments;

F = sensitiveness to heat;

A = sensitiveness to acid scarification

The rating of the species (out of 5), is as follows;

I_{DB} *A. mearnsii* = 4 + 3

= 7

I_{DB} *A. melanoxylon* = 2 + 4

= 6

I_{DB} *P. lophantha* = 3 + 0

= 3

A. mearnsii, being most resistant to heat treatments, *i.e.* which will not be killed rapidly by heat, gets a high score for factor F, whereas *A. melanoxylon* gets a low score because it is killed more rapidly by heat treatments. In the case of factor A, *A. melanoxylon* gets a high score because it will be stimulated to germinate more rapidly by passing through the gut of a bird, but this is unlikely to kill them. *P. lophantha* gets a low score, because it would probably not be taken by birds, and if it is taken, it will have no effect on the dormancy of the seed, *i.e.* it will not be stimulated to germinate or be killed.

The rating of the seeds for I_{DB} therefore, is: 1) *A. mearnsii*, 2) *A. melanoxylon* and 3) *P. lophantha*.

9.4 Seed germination characteristics

A. mearnsii and *A. melanoxylon* have a greater imbibition rate than *P. lophantha*, but this is not reflected in the germination rates, which are more or less the same (Chapter 3). None of the species appears to have a light requirement for germination at temperatures between 10 and 30°C. *P. lophantha* germinates under the widest amplitude of temperatures, followed by *A. mearnsii* and *A. melanoxylon*. *A. melanoxylon* exhibits a very poor (or very slow) germination at 5°C. The hypothesis that *A. melanoxylon* has a narrower ecological amplitude is therefore accepted.

P. lophantha appears to be more sensitive than the two acacias to wetting and drying cycles but there does not appear to be large differences in drought resistance. The sensitivity of *P. lophantha* to desiccation periods could be exploited by stimulating the seeds to germinate in early summer, when soil moisture is low or variable.

The invasive ability in terms of light, moisture and temperature requirements can be expressed as follows:

$$\mathbf{I_{GR} = L + T + M}$$

where

I_{GR} = invasive ability in terms of germination requirements;

L = light requirements;

T = temperature requirements;

M = moisture requirements

The rating of the species (out of 5), is as follows:

I_{GR} *A. mearnsii* = 5 + 4 + 5

= 14

I_{GR} *A. melanoxylon* = 5 + 3 + 4

= 12

I_{GR} *P. lophantha* = 5 + 5 + 3

= 13

The rating of the species in terms of I_{GR} is: 1) *A. mearnsii*, 2) *P. lophantha* and 3) *A. melanoxylon*.

9.5 Establishment and early growth of seedlings

Interesting interactions occurs where seeds of the three species are sown at different depths in different species mixtures (Chapter 5). The seeds can be classified as "powerful" and "weak" according to their ability to establish from deeper sowing depths. Therefore *P. lophantha* seed can be considered "powerful", *A. mearnsii* seed "less powerful" and *A. melanoxylon* seed "weak". The data indicates that "powerful" seed enhance the ability of "weaker" seed to establish from deeper depths while the "weaker" seeds reduce the ability of "powerful" seed to establish from deeper sowing depths. This has implications for control of the seed bank if the density of the seed bank is reduced by, for instance, seed feeding insects. Less dense seed banks will probably be controlled better by a slow, hot burn that kills most of the seed in the upper layers of the seed bank, while the deeper buried seeds might be stimulated to germinate, but might not be able to establish seedlings. This is in particular applicable to species with "weak" seed, such as *A. melanoxylon*. The hypothesis that large-seeded species (*P. lophantha*) will be able to establish from deeper sowing depths than the smaller-seeded species is accepted. The second hypothesis that the small-seeded species will have similar abilities to establish from deeper sowing depths, however, is refuted, implicating that chemical composition of seeds is as important as seed size in determining the establishing ability of the species.

The dry matter production data is hard to interpret, because factors such as the number of seedlings and the time of establishment of the seedlings will probably obscure differences between seedlings of the different species. It is, however, clear that *P. lophantha* seedlings have a significant competitive advantage over the seedlings of the two *Acacia* species, most probably due to the bigger seed size.

Invasive ability in terms of establishment and early growth of seedlings can be calculated as follows:

$$\mathbf{I_{EG} = E + G}$$

where

I_{EG} = invasive ability in terms of seedling establishment and early growth;

E = ability to establish from deeper depths;

G = early growth of seedlings

The species were rated (out of 5) as follows:

I_{EG} *A. mearnsii* = 3 +3

= 6

I_{EG} *A. melanoxylon* = 1 + 3

= 4

I_{EG} *P. lophantha* = 5 + 5

= 10

In terms of I_{EG} the species are ranked as follows: 1) *P. lophantha*, 2) *A. mearnsii* and 3) *A. melanoxylon*.

9.6 Seedling growth as affected by nutrients, drought and shade

The reaction of the seedlings in monoculture to nutrient concentration is confusing (Chapter 6). *P. lophantha* is the only species growing optimally at the 100% nutrient concentration, while growth of *A. mearnsii* is decreased at that concentration and *A. melanoxylon* appears indifferent to nutrient concentrations. *P. lophantha* is the better competitor at all nutrient levels whereas the outcome of the competition between *A. mearnsii* and *A. melanoxylon* is obscured by the erratic growth of *A. mearnsii* seedlings in the glasshouse. The R- and K-values, however, indicate that, in general, *A. mearnsii* has a more negative effect on *A. melanoxylon* than *vice versa*. Hypothesis 3 in Chapter 6 is therefore refuted, because it could not be proven that *A. melanoxylon* is less influenced by nutrient stresses than the other two species.

The shade and moisture stress treatments also yield conflicting results. It does, however, appear as if *A. melanoxylon* is more sensitive to shade than *A. mearnsii*, which in turn is more sensitive than *P. lophantha*. The growth of *P. lophantha* appears to be more severely inhibited by moisture stress. *P. lophantha* again proves to be the better competitor under all moisture and light levels. There is no clear

indication of which of *A. mearnsii* and *A. melanoxylon* is the better competitor under any moisture or light level. Hypothesis 1 (*P.lophantha* and *A. melanoxylon* will be less influenced by shading than *A. mearnsii*) and hypothesis 2 (*P. lophantha* and *A. mearnsii* will be more drought resistant than *A. melanoxylon*), are both refuted.

Judging from the field experiments, however, it appears as if *P. lophantha* seedlings will lose the size advantage at some stage in the future, probably because of its ability to become reproductive within 18 months, which might slow down vegetative growth rate.

The invasive ability of the seedlings in terms of their reaction to light, moisture and nutrient stress is estimated as follows:

$$\mathbf{I_{SG} = L + M + N + C}$$

where

I_{SG} = invasive ability in terms of seedling reaction to light, moisture and nutrient stress;

L = reaction of seedlings to light levels;

M = reaction of seedlings to moisture levels;

N = reaction of seedlings to nutrient levels;

C = overall competitive ability of the seedlings

The species are rated (out of 5) as follows :

I_{SG} *A. mearnsii* = 3 + 4 + 4 + 2

= 13

I_{SG} *A. melanoxylon* = 2 + 4 + 4 + 2

= 12

I_{SG} *P. lophantha* = 5 + 2 + 5 + 5

= 17

The ranking of the species in terms of I_{SG} is: 1) *P. lophantha*, 2) *A. mearnsii* and 3) *A. melanoxylon*.

9.7 Aspects of control

Burning is a valuable tool in reducing the size of the soil stored seed bank of the woody leguminous invaders. It should, however, be applied judiciously to avoid damage to the indigenous vegetation. It has been proposed that invader populations be burned standing to reduce (or, in some cases, increase) the intensity of the fire, as well as reducing the cost of the control programme. This study shows that a standing burn, although it reduces the soil stored seed bank, is not effective as a control measure due to the expansion and increase in size of the aboveground population of *A. mearnsii* and the hypothesis tested in Chapter 7 is therefore refuted. A standing burn of *A. melanoxylon* will probably be even less successful, because of its greater ability to coppice from stems as well as roots. *P. lophantha*, being unable to coppice, might be burned standing, provided that a relatively clean burn is obtained, so that no charred remains hinder the subsequent seedling control. The *P. lophantha* seedlings are relatively easy to kill by means of herbicide application (Pieterse 1994).

This study probes the control of species by utilization (Chapter 8). None of the three species appears to have significant negative (allelopathic) influences on the growth of maize and wheat. They, however, also have no beneficial effect on the crops in terms of addition of nitrogen to the soil. Therefore, the hypothesis that addition of mulch will have no significant effect on crop growth, is accepted, but the results are promising enough to continue the research in the field, where more positive reactions in terms of moisture conservation and weed suppression are expected. Continuous harvesting of leaves and twigs for mulching purposes could be considered as a way of control, because it removes biomass, which in turn will lead to less water consumption and would probably be detrimental to the reproductive capacity.

If the findings of Chapter 7 could be extrapolated to *A. melanoxylon* and *P. lophantha*, an estimate of the effects of similar treatments on these species can be

made and the species can again be ranked according to their susceptibility to those control measures.

The invasive potential in terms of susceptibility to two control methods can be calculated as follows:

$$\mathbf{I_{CM} = B + U}$$

where

I_{CM} = invasive ability based on susceptibility to three control measures;

B = resistance to a standing burn;

U = suitability for utilization as mulch;

The species can then be rated (out of 5) as follows:

I_{CM} *A. mearnsii* = 4 + 2

= 6

I_{CM} *A. melanoxylon* = 5 + 2

= 7

I_{CM} *P. lophantha* = 2 + 4

= 6

P. lophantha does not coppice (Taylor 1978), therefore it would probably not be utilized as a sustainable source of mulch, and is therefore considered "resistant" to this particular control method, hence the high score for the factor U.

The ranking of the species in terms of I_{CM} is: 1) *A. melanoxylon,* followed by *A. mearnsii* and *P. lophantha.*

9.8 Final ranking of species

The decision as to which species is the most invasive, or rather, has the greatest invasive ability, will in this case be based on the invasive ability rankings allocated to

each species in the preceding sections. All the rankings from the different sections will carry the same weight. The overall invasive ability of the species can therefore be estimated by summing the rankings of the species in the preceding sections as follows:

$$I_O = I_{RD} + I_{ST} + I_{DB} + I_{GR} + I_{EG} + I_{SG} + I_{CM}$$

where

I_O = overall invasive ability;

I_{RD} = invasive ability in terms of regenerating capacity and dispersal adaptation;

I_{ST} = invasive ability in terms of seed and testa characteristics;

I_{DB} = invasive ability in terms of sensitiveness to dormancy breaking mechanisms;

I_{GR} = invasive ability in terms of germination requirements;

I_{EG} = invasive ability in terms of establishment and early growth of seedlings;

I_{SG} = invasive ability in terms of seedling growth;

I_{CM} = invasive ability in terms of resistance to control methods;

The overall rating of the species is given in Table 1. According to these rankings it appears as if *A. melanoxylon* is potentially the most invasive of the three species (Table 1). *A. melanoxylon*'s strength appears to be in its ability to find vacant habitats and once it has established itself there, to occupy it successfully. These strengths are due to the production of large quantities of seeds that are adapted to successful long-distance dispersal by birds. The longevity of the trees coupled with their resistance to mechanical control is another strength. Weak points in the life cycle is the inability of seedlings to establish from deeper germination depths and the inability to grow well under shady conditions relative to the other two species.

Table 1 The overall rating of *Acacia mearnsii, A. melanoxylon* and *Paraserianthes lophantha* in terms of invasive ability.

Parameters*	*A. mearnsii*	*A. melanoxylon*	*P. lophantha*
I_{RD}	18	22	10
I_{ST}	22	24	22
I_{DB}	7	6	3
I_{GR}	14	12	13
I_{EG}	6	4	10
I_{SG}	13	12	17
I_{CM}	10	9	9
I_O	90	89	84

*I_{RD} = invasive ability in terms of regenerating capacity and dispersal adaptation;

I_{ST} = invasive ability in terms of seed and testa characteristics;

I_{DB} = invasive ability in terms of sensitiveness to dormancy breaking mechanisms;

I_{GR} = invasive ability in terms of germination requirements;

I_{EG} = invasive ability in terms of establishment and early growth of seedlings;

I_{SG} = invasive ability in terms of seedling growth;

I_{CM} = invasive ability in terms of resistance to control methods;

I_O = overall invasive ability;

The score of *A. mearnsii* is almost similar to those of *A. melanoxylon* (Table 1). If it is taken into consideration that the growth of *A. mearnsii* seedlings are probably inhibited under greenhouse conditions, it could have even higher scores and greater invasive ability than that indicated by the ranking. No obvious weak phases in the life cycle can be detected.

The strengths of *P. lophantha* is its aggressive, fast growing seedlings that can outcompete the other two species during the early life stages, and in its early maturation habit, which allows it to produce seeds within 18 months after establishment of seedlings. Species that produce offspring earlier, usually have a competitive advantage over later seeding species in terms of long term population

growth (Harper 1977). The weak link in the life cycle of *P. lophantha* is probably the susceptibility of the adult trees to control methods. It is probably shorter lived than *A. melanoxylon* and is much easier to control.

9.9 Conclusion

The results of these studies should be interpreted keeping in mind that only seed collected in the vicinity of Stellenbosch was used. It is probable that especially *A. mearnsii* and *A. melanoxylon* occurring in other parts of the country could belong to different races of the same species, because different introductions of *A. mearnsii* (Sherry 1971) and *A. melanoxylon* (Shaughnessy 1980) were made into South Africa. However, although differences in germination characteristics may occur between the seed of different races, I believe that dormancy characteristics of seeds and methods to break dormancy, will basically be similar, irrespective of the race of species involved.

Germination characteristics indicate that all three species will be able to establish under a wide range of environmental conditions and their presence in natural ecosystems should be monitored closely. Once they have established and produced seeds, it will be difficult to eradicate them from such environments.

The fact that *P. lophantha* seeds are not dispersed and buried by ants, indicates that they will normally be buried rather shallowly in the soil seed bank and that fire should therefore be effective in reducing the seed bank. The same principle applies, to a lesser extent, to *A. melanoxylon*, where a small proportion of seeds are taken by ants. A slow fire that heats the soil for an extended period, will cause the most damage to these buried seeds. The finding that dry heat is more detrimental to seeds than wet heat, can be applied by burning in summer in the southwestern Cape when soil moisture is low. A burn under such conditions should destroy most seeds of these two species and the seedlings that do establish, should succumb to drought during a normal summer in the southwestern Cape.

A. mearnsii, on the other hand, should have a bigger proportion of seeds in the deeper soil layers due to burial of the seeds by ants. Therefore it should probably be better to try to stimulate the seeds to germinate because sufficient heat to kill the seeds will seldom reach the depths where the seeds are buried. Wet heat, in other words, burning when the soil is moist, will affect the seeds sooner than dry heat. In the case

of *A. mearnsii*, it will probably be better to burn after the first rains have fallen in autumn, or early in spring when the soil is still moist. The objective will thus be to reduce the soil seed bank by stimulating the seeds to germinate and to concentrate on mortality of the seedlings in the control programme.

It should be kept in mind, that, under certain conditions, it is inadvisable to burn. It has also been shown that autumn burns are less detrimental to the indigenous fynbos vegetation (Seydack & Bekker 1995). The decision to include fire in a control programme will therefore also be dictated by the presence and sensitivity of the indigenous vegetation. Fire as a management tool in control programmes has been criticized (Breytenbach 1989; Macdonald 1991a) and the competitive role that indigenous vegetation play has been emphasised (Macdonald 1991a; Macdonald & Wissel 1992). However, the relatively good ability of these three species to grow under shady conditions, indicate that it will not be a feasible option to rely on existing vegetation or cover crops to suppress establishing seedlings. In the absence of fire, a close watch should be kept on areas where the plants occurred for up to 50 years, due to the longevity of the seed. I believe that, conditions permitting, fire should be used to control the soil seed bank. Once most of the seeds are killed or flushed from the seed bank by a slow fire and the resulting seedlings controlled, chances of more seedlings establishing later on are slim and follow-up operations can be scaled down relative to areas which were not burned.

The burning of standing populations as was suggested for *A. longifolia* by Pieterse (1986) and for *A. cyclops* by Holmes (1989b) is not a viable option for *A. mearnsii* and will probably be even less successful in the case of *A. melanoxylon*, which coppices and suckers more profusely than *A. mearnsii*. The method could, however, be used against *P. lophantha*, which does not coppice and which probably have a relatively shallow seed bank.

Observations in Chapter 7 indicate that *A. mearnsii* seedlings are not effectively suppressed by an existing grass/restio layer after a fire. It is doubtful whether the sowing of grass seeds as advocated by Campbell (1987; 1993) will, on its own, be enough to suppress *A. mearnsii* seedlings. However, if the growth of the legume seedlings can be retarded by application of sub-lethal doses of relatively cheap

hormonal herbicides, oversowing might be a viable proposition. In the southwestern Cape, a better adapted grass species such as *Ehrharta calycina* could be sown if sufficient seed could be collected.

Results from Chapter 5 indicate that lower seed densities in the soil seed bank may reduce the probability of seedlings establishing from deeper germination depths. Reduction of seed production by biological control agents will probably have a bigger effect on seedling establishment from soil seed banks in *A. mearnsii* than in other species. *A. mearnsii*, which has ant-dispersed seeds, will probably have a larger proportion of seeds in the deeper soil layers than the other two species. The smaller the amount of seeds produced, the bigger the proportion of the seeds that will be buried by the ants, provided that density-dependent effects will not result in the ants collecting less seeds. If, however, the density of the soil stored seed decline to a certain threshold level, seedlings will find it increasingly difficult to establish from the deeper germination depths.

The following recommendations could possibly be of use to the clearing teams of the RDP-funded "Work for water " programme. If fire cannot be used, felled plants should, according to their sprouting habit, be treated by applying registered herbicides to the cut stumps. The fungus *Cylindrobasidium laeve*, that kills resprouting stumps of *A. mearnsii*, could also be applied as a mycoherbicide on *A. mearnsii* stumps (Morris 1995). Branches should be stacked to cover the area inside the dripline of the tree to provide maximum shading conditions and wildfires should be kept out of the area as far as possible. Annual follow-up programmes to control seedling regeneration should be continued for 20-50 years due to the longevity of the seeds.

Where fire can be applied, plants should be felled and the branches stacked around the stumps where trees occur singly, or where dense stands occur, branches should be distributed evenly over the area. Sufficient time should be allowed for the felled material to dry out and for the stumps to coppice before the area is burned. Burning of the coppicing stumps might possibly deplete the reserves of the stumps to such an extent that some might be killed, in which case less stump-applied herbicides will be necessary. The burning should be carried out in the appropriate season as discussed above and a grass cover crop might be sown to suppress the seedlings. Strong

growing indigenous shrubs such as *Chrysanthemoides monilifera* or *Metalasia muricata* might also be used. The seedlings can then be controlled manually if possible or by means of herbicide application. Stumps that are still alive after the fire, should also be treated with the appropriate herbicides.

This study, as often happens, creates as many questions as it provides answers. The study indicates that there might be a relationship between mode of dispersal, cell wall anatomy and reaction to different dormancy-breaking mechanisms (*i.e.* heat or acid). From an ecological point of view, it would be interesting to determine if such a relationship exists by including a larger number of ant- and bird-dispersed legume species. The invasive ability rankings of the species in this study are but an estimation. The real proof will be in long term population dynamics studies of the three species, as well as in seedling competition studies under natural conditions. Seed bank studies such as those carried out by Holmes (1989a) can also be useful in terms of assessing control options. The assumption made in this discussion that the profile of the soil seed bank of ant-dispersed species will differ from those of non-ant-dispersed species, should be investigated. The efficacy of a standing burn treatment to control *P. lophantha* infestations, should also be investigated, as it could mean a substantial saving on labour. The effect of fire on coppicing stumps is also a worthwhile project to investigate, as it may mean savings on herbicide. The mistake often made of not treating stumps of coppicing species, should be discouraged, because it escalates follow-up costs (Macdonald & Wissel 1992). It is therefore imperative that the most economical way of treating coppicing stumps be determined.

Phyllode acacias are generally harder to control chemically than true-leafed acacias (Donald & Kirby-Smith 1983). *A. mearnsii* and *P. lophantha* are relatively easy to control chemically (Campbell 1993, Pieterse 1994) but no herbicide has been registered against *A. melanoxylon* (Vermeulen *et al.* 1993). A factor such as time of application can play a major role in the efficacy of herbicides on phyllode acacias (Pieterse & Mcdermott 1994), and should be investigated in the case of *A. melanoxylon*. The use of oversown crops in conjunction with judiciously applied herbicides to control seedlings should also be investigated.

This study provides some insights into the biology of these particular species. This knowledge is useful in the planning of effective control programmes and should be made available to managers who should benefit from it.

Chapter 10

References

AARSSEN, L. W. 1989. Competitive ability and species coexistence: a "plant's - eye" view. *Oikos* 56, 386-401.

ABBOTT, I. 1984. Emergence, early survival and growth of seedlings of six tree species in mediterranean forests of Western Australia. *For. Ecol. Manage.* 9, 51-66.

ANON. 1961. Use of black wattle in arable crop rotations. *Rep. Wattle Res. Inst.* for 1960-1961, 18-19.

ANON. 1963. Use of wattle in arable crop rotations. *Rep. Wattle Res. Inst.* for 1962-1963, 19-21.

AOAC. 1984. Official methods of analysis. 14th ed. Washington, D.C.: Assoc. of Official Analytical Chemists.

AUGSPURGER, C.K. 1984. Light requirements of neotropical tree seedlings: a comparative study of growth and survival. *J. Ecol.* 72, 777-795.

AULD, T.D. 1986a. Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd: dispersal and the dynamics of the soil seed bank. *Aust. J. Ecol.* 11, 235-254.

AULD, T.D. 1986b. Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd: fire and the transition to seedlings. *Aust. J. Ecol.* 11, 373-385.

AUSTIN, M.P., FRESCO, L.F.M., NICHOLLS, A.O., GROVES, R.H. & KAYE, P.E. 1988. Competition and relative yield: estimation and interpretation at different densities and under various nutrient concentrations using *Silybum marianum* and *Cirsium vulgare*. *J. Ecol.* 76, 157-171.

BAKER, H.G. 1972. Seed weight in relation to environmental conditions in California. *Ecology* 53, 697-1010.

BAZZAZ, F.A. & HARPER, J.L. 1976. Relationship between plant weight and numbers in mixed populations of *Sinapsis alba* (L.) Rabenh. and *Lepidium sativum* L. *J. Appl. Ecol.* 13, 211-216.

BEARD, J.S. 1983. Ecological control of the vegetation of south-western Australia: moisture versus nutrients. In: Mediterranean-type ecosystems. The role of

nutrients, eds. F.J. Kruger, D.T. Mitchell & J.U.M. Jarvis. pp. 66-73. Ecological studies Vol. 43. Springer-Verlag, Berlin.

BEARD, S.J. 1956. Results of the Mountain Home rainfall interception and infiltration project in Black Wattle, 1953/54. *J. S. Afr. For. Assoc.* 27, 72-86.

BEARD, S.J. 1963. Soil moisture studies using the Berkeley fibreglass instrument. *S. Afr. Forestry J.* 44, 24-28.

BEARD, S.J. & DARBY, G.D. 1951. An experiment on burning in wattle silviculture. *J. S. Afr. For. Assoc.* 20, 53-77.

BELL, D.T. 1994. Interaction of fire, temperature and light in the germination response of 16 species from the *Eucalyptus marginata* forest of south-western Australia. *Aust. J. Bot.* 42, 501-509.

BELL, W.E. & VAN STADEN, J. 1993. Seed structure and germination of *Dichrostachys cinerea*. *S. Afr. J. Bot.* 59, 9-13.

BERG, R.Y. 1981. The role of ants in seed dispersal in Australian lowland heathland. In: Heathlands and related shrublands of the world, Vol. 13, ed. R.L. Specht. pp. 51-58. Elsevier, Amsterdam.

BERRIE, A.M. & DRENNAN, D.S.H. 1971. The effect of hydration-dehydration on seed germination. *New Phytol.* 70, 135-142.

BEVERIDGE, J.L. & WILSIE, C.P. 1959. Influence of depth of planting, seed size and variety on emergence and seedling vigor in alfalfa. *Agron. J.* 51, 731-734.

BEWLEY, J.D. & BLACK, M. 1994. Seeds. Physiology of development and germination. Second edition, Plenum Press, New York.

BI, H. & TURVEY, N.D. 1994. Inter-specific competition between seedlings of *Pinus radiata*, *Eucalyptus regnans* and *Acacia melanoxylon*. *Aust. J. Bot.* 42, 61-70.

BLACK, J.N. 1955. The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (*Trifolium subterranean* L.). *Aust. J. Agric. Res.* 7, 98-108.

BLACK, J.N. 1958. Competition between plants of different initial seed sizes in swards of subterranean clover (*Trifolium subterranean* L.) with particular reference to leaf area and the light microclimate. *Aust. J. Agric. Res.* 9, 299-318.

BLACK, J.N. & WILKINSON, G.N. 1963. The role of time of emergence in determining the growth of individual plants in swards of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Agric. Res.* 14, 628-638.

BOND, P. & GOLDBLATT, P. 1984. Plants of the Cape Flora: a descriptive catalogue. *Jl S. Afr. Bot.* Supplementary Vol. 13.

BORENS, F.M.P. & POPPI, D.P. 1990. The nutritive value for ruminants of Tagasaste (*Chamaecytisus palmensis*), a leguminous tree. *Anim. Feed Sci. Tecnol.* 28, 275-292.

BOSCH, J.M. 1979. Treatment effects of annual and dry period streamflow at Cathedral Peak. *S. Afr. Forestry J.* 75, 9-17.

BOSCH, J.M. & HEWLETT, J.D. 1982. A review of catchment experiments to determine the effect of vegetation changes on water yield and evaporation. *J. Hydrol.* 55, 3-23.

BOTHA, F.C., GROBBELAAR, N. & SMALL, J.G.C. 1984. The effect of water stress on the germination of *Citrullus lanatus* seeds. *S. Afr. J. Bot.* 3, 111-114.

BOUCHER, C. 1978. Black Wattle. In: Plant invaders, beautiful but dangerous, ed. C.H. Stirton. pp. 48-51. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape Town.

BRADSTOCK, R.A. & AULD, T.D. 1995. Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire management in south-eastern Australia. *J. Appl. Ecol.* 32, 76-84.

BRECKE, B.J. & DUKE, W.B. 1980. Dormancy, germination and emergence characteristics of fall panicum (*Panicum dichotomiflorum*) seed. *Weed Sci.* 28, 683-685.

BREYTENBACH, G.J. 1989. Alien control: can we afford to slash and burn *Hakea* in Fynbos ecosystems? *S. Afr. Forestry J.* 151, 6-16.

CAMPBELL, P.L. 1987. The control of black wattle. *Weeds* A.24.1. Farming in South Africa, Government Printer, Pretoria.

CAMPBELL, P.L. 1993. Wattle control. Plant Protection Research Institute Handbook No. 3. Plant Protection Research Institute, Pretoria.

CARTER, R.E. & KLINKA, K. 1992. Variation in shade tolerance of Douglas fir, western hemlock and western red cedar in coastal British Columbia. *For. Ecol. Manage*. 55, 87-105.

CASAL, J.F., REIGOSA, M.J. & CARBALLEIRA, A. 1985. Potential allélopathique de *Acacia dealbata* Link . *Rev. Écol. Biol. Sol*. 22, 1-12.

CHAPMAN, R.A., SCOTT, D.F. & LE MAITRE, D. 1995. Hydrological impacts of aliens in Fynbos catchments. In: Managing fynbos catchments for water, eds. C. Boucher, & C. Marais, Proceedings of a workshop held on 30 November 1993 at Stellenbosch, South Africa. *FRD Programme Report Series* 24, 40-43.

CLEMENS, J., JONES, P.G. & GILBERT, N.H. 1977. Effect of seed treatments on germination in *Acacia*. *Aust. J. Bot*. 25, 269-276.

COETSEE, J. 1990. Maniere om wattel te bestry. *Landbouweekblad* 635, 25 Mei 1990, 62-65.

CONSTANTINIDES, M. & FOWNES, J.H. 1993. Nitrogen mineralization patterns of leaf-twig mixtures from tropical leguminous trees. *Agroforestry Syst*. 24, 223-231.

CORCHETE, F. & GUERRA, H. 1986. Effect of NaCl and polyethylene glycol on solute content and glycosidase activities during germination of lentil seeds. *Plant Physiol*. 66, 316-320.

COSTERMANS, L. 1981. Native trees and shrubs of south-eastern Australia. Rigby, Adelaide.

COWLING, R.M., MOLL, E.J. & CAMPBELL, B.M. 1976. The ecological status of the understorey communities of pine forests on Table Mountain. *S. Afr. Forestry J*. 99, 13-24.

CRAWLEY, M.J. 1983. Herbivory, the dynamics of animal-plant interactions. Studies in Ecology 10. Blackwell Scientific Publications, Oxford.

CUNNINGHAM, T.M. & CREMER, R.W. 1965. Control of the understorey in wet *Eucalyptus* forests. *Aust. For*. 29, 4-14.

DAVIDSON, D.W. & MORTON, S.R. 1984. Dispersal adaptations of some *Acacia* species in the Australian arid zone. *Ecology*. 65, 1038-1051.

DEACON, J. 1986. Human settlement in South Africa and archaeological evidence for alien plants and animals. In: The ecology and management of biological

invasions in southern Africa, eds. I.A.W. Macdonald, F.J. Kruger & A.A. Ferrar. Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 3-19. Oxford University Press, Cape Town.

DEAN, S.J., HOLMES, P.M. & WEISS, P.W. 1986. Seed biology of invasive alien plants in South Africa and Namibia. In: The ecology and management of biological invasions in southern Africa, eds. I.A.W. Macdonald, F.J. Kruger & A.A. Ferrar, Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 295-303. Oxford University Press, Cape Town.

DE LANGE, J.H. & BOUCHER, C. 1993. Autecological studies on *Audouinia capitata* (Bruniaceae). 8. Role of fire in regeneration. *S. Afr. J. Bot.* 59, 188-202.

DELL, B. 1980. Structure and function of the strophiolar plug in seeds of *Albizia lophantha*. *Amer. J. Bot.* 67, 556-563.

DENNILL, G.B. 1987. Establishment of the gall wasp *Trichilogaster acaciaelongifoliae* (Pteromalidae) for the biological control of the weed *Acacia longifolia* in South Africa. *Agric. Ecosystems Environ.* 19, 155-168.

DENNILL, G.B. & DONNELLY, D. 1991. Biological control of *Acacia longifolia* and related weed species (Fabaceae) in South Africa. *Agric. Ecosystems Environ.* 37, 115-135.

DE VILLIERS, A.J., VAN ROOYEN, M.W., THERON, G.K. & VAN DEVENTER, H.A. 1994. Germination of three Namaqualand pioneer species, as influenced by salinity, temperature and light. *Seed Sci. & Technol.* 22, 427-433.

DE WITT, C.T. 1960. On competition. *Versl. Landbouwkd. Onderz.* 66, 1-82.

DONALD, D.G.M. & KIRBY-SMITH, J.H. 1983. Chemical control of phyllode Acacias in the S.W. Cape. In: Proc.of the Fifth National Weeds Conf. of South Africa, eds. S. Neser & A.L.P. Cairns. pp.315-326. Stellenbosch 1983.

DONNELLY, D. 1995. Good news for blackwood control. *Plant Protection News* 41, 4.

DRAKE, W.E. 1981. Ant-seed interaction in dry sclerophyll forest on North Stradbroke Island, Queensland. *Aust. J. Bot.* 29, 293-309.

DUKE, S.H. & KAKEFUDA, G. 1981. Role of the testa in preventing cellular rupture during imbibition of legume seeds. *Physiol.* 67, 449-456.

DYE, P.J. & POULTER, T. 1995. A field demonstration of the effects on streamflow of clearing invasive pines and wattle trees from a riparian zone. *S. Afr. Forestry J.* 173, 27-30.

EASTIN, E.F. 1984. Drummond rattlebox (*Sesbania drummondii*) germination as influenced by scarification, temperature, and seeding depth. *Weed Sci.* 32, 223-225.

EISSENSTAT, D.M. & CALDWELL, M.M. 1988. Competitive ability is linked to rates of water extraction. A field study of two aridland tussock grasses. *Oecologia* 75, 1-7.

ELLIS. F.I.B. 1979. Agronomic problems from straw residues with particular reference to reduced cultivation and direct drilling in Britain. In: Straw decay and its effect on disposal and utilization, ed. E. Grossbard. pp. 11-20. John Wiley & Sons, New York.

EMMERICH, W.E. & HARDEGREE, S.P. 1991. Seed germination in polyethylene glycol solution: effects of filter paper exclusion and water vapor loss. *Crop Sci.* 31, 454-458.

FARRELL, T.P. & ASHTON, D.H. 1978. Population studies on *Acacia melanoxylon* R. Br. I. Variation in seed and vegetative characteristics. *Aust. J. Bot.* 26, 365-379.

FEHR, W.R., BURRIS, J.S. & GILLMAN, D.F. 1973. Soybean emergence under field conditions. *Agron. J.* 65, 740-742.

FENNER, M. 1983. Relationships between seed weight, ash content and seedling growth in twenty-four species of Compositae. *New Phytol.* 95, 697-706.

FENNER, M. 1985. Seed ecology. Chapman & Hall, London.

FIRBANK, L.G. & WATKINSON, A.R. 1985. On the analysis of competition within two-species mixtures of plants. *J. Ecol.* 75, 871-886.

FITTER, A.H. & HAY, R.K.M. 1987. Environmental physiology of plants. Academic Press, London.

FLOYD, A.G. 1966. Effect of fire upon weed seeds in the wet sclerophyll forest of northern N.S.W. *Aust. J. Bot.* 14, 143-156.

FLOYD, A.G. 1976. Effect of burning on regeneration from seeds in wet sclerophyll forest. *Aust. For.* 39, 210-220.

FORCELLA, F. 1985. Final distribution is related to rate of spread in alien weeds. Weed Res. 25, 181-191.

FYFIELD, T.P. & GREGORY, P.J. 1989. Effects of temperature and water potential on germination, radicle elongation and emergence of mungbean. *J. Exp. Bot.* 40, 667-674.

GELDENHUYS, C.J. 1986. Costs and benefits of the Australian blackwood *Acacia melanoxylon* in South African forestry. In: The ecology and management of biological invasions in southern Africa, eds. I.A.W. Macdonald, F.J. Kruger and A.A. Ferrar. Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 275-283. Oxford University Press, Cape Town.

GELDENHUYS, C.J., LE ROUX, P.J. & COOPER, K.H. 1986. Alien invasives in evergreen forest. In: The ecology and management of biological invasions in southern Africa, eds. I.A.W. Macdonald, F.J. Kruger and A.A. Ferrar. Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 119-131. Oxford University Press, Cape Town.

GLEADOW, R.M. & ROWAN, K.S. 1982. Invasion by *Pittosporum undulatum* of the forests of Central Victoria. III. Effects of temperature and light on growth and drought resistance. *Aust. J. Bot.* 30, 347-357.

GLYPHIS, J.P., MILTON, S.J. & SIEGFRIED, W.R. 1981. Dispersal of *Acacia cyclops* by birds. *Oecologia* 48, 138-141.

GÖRGENS, A.H.M., LARSEN, E.J. & LITTLE, P.R. 1995. Potential effect of alien vegetation on water resources: a case study of the Riviersonderend System (Theewaterskloof Dam Catchment). In: Managing fynbos catchments for water. eds. C. Boucher & C. Marais. Proceedings of a workshop held on 30 November 1993 at Stellenbosch, South Africa. *FRD Programme Report Series No.* 24, 44-55.

GOVERNMENT GAZETTE. 1984. Conservation of Agricultural Resources Act, No. 43 of 1983. Government Printer, Pretoria.

GRAAFF, J.L. & VAN STADEN, J. 1983a. The effect of different chemical and physical treatments on seed coat structure and seed germination of *Sesbania* species. *Z. Planzenphysiol. Bd.* 112, S. 221-230.

GRAAFF, J.L. & VAN STADEN, J. 1983b. The germination characteristics of two *Sesbania* species. *S. Afr. J. Bot.* 3, 59-62.

GRIME, J.P. & JEFFREY, D.W. 1965. Seedling establishment in vertical gradients of sunlight. *J. Ecol.* 53, 621-642.

GROSS, K.L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *J. Ecol.* 72, 369-387.

GUPTA, B.N. & THAPLIYAL, R.C. 1974. Presowing treatment of black wattle (*Acacia mearnsii* de Willd.) and Australian blackwood (*Acacia melanoxylon* R. Br.) seed. *Indian For.* 100, 733-735.

GUTTERIDGE, R.C. 1992. Evaluation of the leaf of a range of tree legumes as a source of nitrogen for crop growth. *Expl. Agric.* 28, 195-202.

HADAS, A. 1976. Water uptake and germination of leguminous seeds under changing external water potential in osmotic solutions. *J. Exp. Bot.* 98, 480-489.

HAGGAR, J.P. 1994. Trees in alley cropping: competitors or soil improvers? *Outlook Agric.* 23, 27-32

HALLSWORTH, E.G. 1958. Nutrition of legumes. Butterworths, London.

HAMPSON, C.R. & SIMPSON, G.M. 1990a. Effect of temperature, salt, and osmotic potential on early growth of wheat (*Triticum aestivum*). I. Germination. *Can. J. Bot.* 68, 524-528.

HAMPSON, C.R. & SIMPSON, G.M. 1990b. Effect of temperature, salt, and osmotic potential on early growth of wheat (*Triticum aestivum*). II. Early seedling growth. *Can. J. Bot.* 68, 529-532.

HANDAYANTO, E., CADISH, G. & GILLER, K.E. 1994. Nitrogen release from prunings of legume hedgerow trees in relation to quality of the prunings and incubation method. *Plant Soil* 160, 237-248.

HANNA, P.J. 1984. Anatomical features of the seed coat of *Acacia kempeana* (Mueller) which relate to increased germination rate induced by heat treatment. *New Phytol.* 96, 23-29.

HARDEGREE, S.P. & EMMERICH, W.E. 1994. Seed germination response to polyethylene glycol solution depth. *Seed Sci. & Technol.* 22, 1-7.

HARPER, J.L. 1977. Population biology of plants. Academic Press, London.

HARPER, J.L. & OBEID, M. 1967. Influence of seed size and depth of sowing on the establishment and growth of varieties of fibre and oil seed flax. *Crop Sci.* 7, 527-532.

HENDERSON, L. 1989. Invasive alien woody plants of Natal and the North-eastern Orange Free State. *Bothalia* 19, 237-261.

HENDERSON, L. 1991. Invasive alien woody plants of the Orange Free State. *Bothalia* 21, 73-89.

HENDERSON, L. 1992. Invasive alien woody plants of the eastern Cape. *Bothalia* 22, 119-143.

HENDERSON, L. & MUSIL, K.J. 1984. Exotic woody plant invaders of the Transvaal. *Bothalia* 15, 297-313.

HENDERSON, M., FOURIE, D.M.C., WELLS, M.J. & HENDERSON, L. 1987. Declared weeds and alien invasive plants in South Africa. Bulletin 413, Department of Agriculture & Water Supply, Republic of South Africa. Government Printer, Pretoria.

HENDRIX, S.D. & TRAPP, E.J. 1992. Population demography of *Pastina sativa* (Apiaceae): effect of seed mass on emergence, survival and recruitment. *Amer. J. Bot.* 79, 365-375.

HENDRIX, S.D., NIELSEN, E., NIELSEN, T. & SCHUTT, M. 1991. Are seedlings from small seeds always inferior to seedlings from large seeds? Effects of seed biomass on seedling growth in *Pastina sativa* L. *New Phytol.* 119, 299-305.

HENDRY, N.S. & VAN STADEN, J. 1982. Seed germination and the potential for control of *Acacia mearnsii* as a weed. *S. Afr. J. Sci.* 78, 206-207.

HEYDECKER, W. 1973. Appendix 3. Glossary of terms. In: Seed ecology, ed. W. Heydecker, pp. 553-557. Butterworths, London.

HILL, M.J. & LUCK, R. 1991. The effect of temperature on germination and seedling growth of temperate perennial pasture legumes. *Aust. J. Agric. Res.* 42, 175-189.

HODGKINSON, K.C. & OXLEY, R.E. 1990. Influence of fire and edaphic factors on germination of the arid zone shrubs *Acacia aneura, Cassia nemophila* and *Dodonaea viscosa*. *Aust. J. Bot.* 38, 269-279.

HOLMES, P.M. 1989a. A comparative study of the seed bank dynamics of two congeneric alien invasive species. Ph.D Thesis, Faculty of Science, University of Cape Town. 1989.

HOLMES, P.M. 1989b. Effects of different clearing treatments on the seed-bank dynamics of an invasive Australian shrub, *Acacia cyclops*, in the Southwestern Cape, South Africa. *For. Ecol. Manage.* 28, 33-46.

HOLMES, P.M., MACDONALD, I.A.W. & JURITZ, J. 1987. Effects of clearing treatment on seed banks of the alien invasive shrubs *Acacia saligna* and *Acacia cyclops* in the southern and south-western Cape, South Africa. *J. Appl. Ecol.* 24, 1045-1051.

HOROWITZ, M. & TAYLORSON, R.B. 1984. Hardseededness and germinability of Velvetleaf (*Abutilon theophrasti*) as affected by temperature and moisture. *Weed Sci.* 32, 111-115.

HOVELAND, C.S. & BUCHANAN G.A. 1973. Weed seed germination under simulated drought. *Weed Sci.* 21, 322-324.

HUCL, P. 1993. Effects of temperature and moisture stress on the germination of diverse common bean genotypes. *Can. J. Plant Sci.* 73, 697-702.

HUFFAKER, C.B. 1964. Fundamentals of biological weed control. In: Biological control of insect pests and weeds, ed. P. de Bach. pp. 631-649. Chapman and Hall, London.

HUGHES, C.E. & STYLES, B.T. 1989. The benefits and risks of woody legume introduction. In: Advances in legume biology, eds. C.H. Stirton & J.L. Zaruchi. *Monogr. Syst. Bot. Missouri. Bot. Gar.* 29, 505-531.

HUNTER, J.R. & ERIKSON, A.E. 1952. Relation of seed germination to soil moisture tension. *Agron. J.* 44, 107-109.

JANSEN, P.I. & ISON, R.L. 1994a. Hydration-dehydration and subsequent storage effects on seed of the self-regenerating annuals *Trifolium balansae* and *T. resupinatum*. *Seed Sci. & Technol.* 22, 435-447.

JANSEN, P.I. & ISON, R.L. 1994b. Temperature effects on germination of *Trifolium balansae* and *T. resupinatum* with special reference to high-temperature dormancy. *Aust. J. Agric. Res.* 45, 689-701.

JEFFERY, D.J., HOLMES, P.M. & REBELO, A.G. 1988. Effects of dry heat on seed germination in selected indigenous and alien legume species in South Africa. *S. Afr. J. Bot.* 54, 28-34.

JOHNSTONE, S.K., WALKER, R.H. & MURRAY, D.S. 1979. Germination and emergence of Hemp Sesbania (*Sesbania exaltata*). *Weed Sci.* 27, 290-293.

JOLIFFE, P.A., MINJAS, A.N. & RUNECKLES, V.C. 1984. A reinterpretation of yield relationships in replacement series experiments. *J. Appl. Ecol.* 21, 227-243.

JONES, R.M. 1963. Studies in the autecology of the Australian acacias in South Africa. V. Preliminary studies of the germination of seed of *Acacia cyclops* and *Acacia cyanophylla*. *S. Afr. J. Sci.* 59, 296-298.

JONES, R.M., ROUX, E.R. & WARREN, J.M. 1963. Studies in the autecology of Australian acacias in South Africa. 4. The production of toxic substances by *Acacia cylops* and *A. cyanophylla* and their possible ecological significance. *S. Afr. J. Sci.* 59, 295-296.

JORDAAN, L. & VAN ZYL, D. 1976. Maak plan met dié boom. *Landbouweekblad* 15, 19-23. 16 th April, 1976.

KACHAKA, S., VANLAUWE, B. & MERCKX, R. 1993. Decomposition and nitrogen mineralization of prunings of different quality. In: Soil organic matter dynamics and sustainability of tropical agriculture, eds. K. Mulongoy & R. Merckx. pp. 199-208. Wiley-Sayce Co-Publication, Leuven.

KANG, B.T., REYNOLDS, L. & ATTA-KRAH, A.N. 1990. Alley farming. *Adv. Agron.* 43, 315-359.

KAUFMAN, M.R. 1969. Effects of water potential on germination of lettuce, sunflower and citrus seeds. *Can. J. Bot.* 47, 1761-1764.

KELLEY, K.M. & VAN STADEN, J. 1985. Effect of acid scarification on seed coat structure, germination and seedling vigour of *Aspalathus linearis*. *J. Plant Physiol.* 121, 37-45.

KELLEY, K.M., VAN STADEN, J. & BELL, W.E. 1992. Seed coat structure and dormancy. *Plant Growth Regulation* 11, 201-209.

KIEPE, P. & RAO, M.R. 1994. Management of agroforestry for the conservation and utilization of land and water resources. *Outlook Agric.* 23, 17-25.

KLUGE, R.L., ZIMMERMANN, H.G., CILLIERS, C.J. & HARDING, G.B., 1986. Integrated control for invasive alien weeds. In: The ecology and management of biological invasions in Southern Africa, eds. I.A.W. Macdonald, F.J. Kruger & A.A. Ferrar. Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 295-303. Oxford University Press, Cape Town.

KOLB, T.E. & STEINER, K.C. 1990. Growth and biomass partitioning response of northern red oak genotypes to shading and grass root competition. *For. Sci.* 36, 293-303.

KOLB, T.E., STEINER, K.C., McCORMICK, L.H. & BOWERSOX, T.W. 1990. Growth response of Northern red oak and yellow poplar seedlings to light, soil moisture and nutrients in relation to ecological strategy. *For. Ecol. Manage.* 36, 65-78.

LAL, R. 1991. Myths and scientific realities of agroforestry as a strategy for sustainable management for soils in the tropics. *Adv. Soil Sci.* 15, 91-137.

LAMBERT, M.G., JUNG, G.A., HARPSTER, H.W., BUDDING, P.J. & WEWALA, G.S. 1989. Forage shrubs in North Island hill country 3. Forage digestibility. *N. Z. J. Agric. Res.* 32, 491-497.

LAW, R. & WATKINSON, A.R. 1987. Response-surface analysis of two-species competition: an experiment on *Phleum arenarium* and *Vulpia fasciculata*. *J. Ecol.* 75, 871-886.

LE MAITRE, D., BOSCH, J. & LESCH, W. 1995. Management of riparian zones for water yield and quality. In: Managing fynbos catchments for water, eds. C. Boucher & C. Marais. Proceedings of a workshop held on 30 November 1993 at Stellenbosch, South Africa. *FRD Programme Report Series No.* 24, 88-98.

LEPART, J. & DE BUSSCHE, M. 1991. Invasion processes as related to succession and disturbance. In: Biogeography of mediterranean invasions, eds. R.H. Groves & F. Di Castri, pp. 199-177. Cambridge University Press, Cambridge.

LEVIN, D.A. 1974. The oil content of seeds : an ecological perspective. *Amer. Nat.* 108, 193-206.

LITTLE, P.R. 1995. Water resources of the Western Cape. In: Managing fynbos catchments for water, eds. C. Boucher & C. Marais. Proceedings of a workshop held on 30 November 1993 at Stellenbosch, South Africa. *FRD Programme Report Series No* 24, 3-8.

LOW, A.B & REBELO, A.G. (Eds) 1996. Vegetation of SouthAfrica, Lesotho and Swaziland. Dept. Environmental Affairs & Tourism, Pretoria.

LÜCKHOFF, H.A. 1977. Comment on A.C.A.C.I.A. *S. Afr. Forestry J.* 102, 15-16.

MACDONALD, I.A.W. 1984. Is the Fynbos Biome especially susceptible to invasion by alien plants? A re-analysis of available data. *S. Afr. J. Sci.* 80, 369-377.

MACDONALD, I.A.W. 1991a. Chopping out aliens - with a computer. Farmers Weekly 81052, December 27, 42-45.

MACDONALD, I.A.W. 1991b. Conservation implications of the invasion of Southern Africa by alien organisms. Ph.D thesis, Faculty of Science, University of Cape Town, Cape Town.

MACDONALD, I.A.W. & JARMAN, M.L. (Eds.) 1984. Invasive alien organisms in the terrestrial ecosystems of the Fynbos Biome, South Africa. *South African National Scientific Programmes Report No.* 85.

MACDONALD, I.A.W. & JARMAN, M.L. (Eds.). 1985. Invasive alien plants in the terrestrial ecosystems of Natal, South Africa. *South African National Scientific Programmes Report No.* 118.

MACDONALD, I.A.W. & RICHARDSON, D.M. 1986. Alien species in terrestrial ecosystems of the Fynbos Biome. In: The ecology and management of biological invasions in southern Africa, eds. I.A.W. Macdonald, F.J. Kruger & A.A. Ferrar. Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 77-91. Oxford University Press, Cape Town.

MACDONALD, I.A.W. & WISSEL, C. 1992. Determining optimal clearing treatments for the alien invasive shrub *Acacia saligna* in the southwestern Cape, South Africa. *Agric. Ecosystems Environ.* 39, 169-186.

MACDONALD, I.A.W., JARMAN, M.L. & BEESTON, P. (Eds.). 1985. Management of invasive alien plants in the fynbos biome. *South African National Scientific Programmes, Report No.* 111.

MANNING, J.C. & VAN STADEN, J. 1987. The role of the lens in seed imbibition and seedling vigour of *Sesbania punicea* (Cav.) Benth. (Leguminosae:Papilionoideae). *Ann. Bot.* 59, 705-713.

MARTIN, R.E., MILLER, R.L. & CUSHWA, C.T. 1975. Germination response of legume seeds subjected to moist and dry heat. *Ecology* 56, 1441-1445.

MAYER, A.M. & POLJAKOFF-MAYBER, A. 1982. The germination of seeds. Pergamon Press, Oxford.

McCULLAGH, P. & NELDER, J.A. 1983. Generalized linear models. Chapman and Hall, London.

MCDOWELL, C.R. & MOLL, E.J. 1981. Studies of seed germination and seedling competition in *Virgilia oroboides* (Berg.) Salter, *Albizia lophantha* (Willd.) Benth. and *Acacia longifolia* (Andr.) Willd. *Jl S. Afr. Bot.* 47, 653-658.

McKERSIE, B.D. & STINSON, R.H. 1980. Effect of dehydration on leakage and membrane structure in *Lotus corniculatus* seeds. *Plant Physiol.* 66, 316-320.

MILEWSKI, A.V. & BOND, W.J. 1982. Convergence of myrmecochory in mediterranean Australia and South Africa. In: Ant-plant interactions in Australia, ed. R.C. Buckley, pp. 89-98. Dr. W. Junk Publishers, The Hague.

MILTON, S.J. 1980a. Australian acacias in the south-western Cape: pre-adaptation, predation and success. In: Proc. 3rd National Weeds Conference of South Africa, Eds. S. Neser & A.L.P. Cairns, pp. 69-78. Stellenbosch.

MILTON, S.J. 1980b. Studies on Australian *Acacias* in the south-western Cape, South Africa. M.Sc thesis, University of Cape Town, Cape Town.

MILTON, S.J. & HALL, A.V. 1981. Reproductive biology of Australian Acacias in the south-western Cape Province, South Africa. *Trans. roy. Soc. S. Afr.* 44, 465-487.

MOLL, E.J. 1978. Blackwood. In: Plant invaders, beautiful but dangerous, ed. C.H. Stirton. pp. 52-55. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape Town.

MOLL, E.J. & BOSSI, L. 1984. A current assessment of the extent of the natural vegetation of the Fynbos Biome. *S. Afr. J. Sci.* 80, 355-358.

MOLL, E.J., MCKENZIE, B. & MCLACHLAN, D. 1980. A possible explanation for the lack of trees in the fynbos, Cape Province, South Africa. *Biol. Conserv.* 17, 221-228.

MONK, D., PATE, J.S. & LONERAGAN, W.A. 1981. Biology of *Acacia pulchella* R. Br. with special reference to symbiotic nitrogen fixation. *Aust. J. Bot.* 29, 579-592.

MOORE, R.P. 1973. Tetrazolium staining for assessing seed quality. In: Seed ecology, ed. W. Heydecker, pp. 347-366. Butterworths, London.

MORAN, V.C. & HOFFMANN, J.H. 1989. The effect of herbivory by a weevil species, acting alone and unrestrained by natural enemies, on growth and phenology of the weed *Sesbania punicea*. *J. Appl. Ecol.* 26, 967-977.

MORRIS, M.J. 1995. Resprouting of black wattle stumps - a solution in sight. *Plant Protection News* 42, 4-5.

MORRISON, D.A., AULD, T.D., RISH, S., PORTER, C. & McCLAY, K. 1992. Patterns of testa-imposed seed dormancy in native Australian legumes. *Ann. Bot.* 70, 157-163.

MUSIL, C.F. 1993. Effect of invasive Australian acacias on the regeneration, growth and nutrient chemistry of South African lowland fynbos. *J. Appl. Ecol.* 30, 361-372.

NANNI, U.M. 1970. Trees, water and perspective. *S. Afr. Forestry J.* 75, 9-17.

NG, F.S.P. 1978. Strategies of establishment in Malayan forest trees. In: Tropical trees as living systems, eds. P.B. Tomlinson & M.H. Zimmerman. pp. 129-162. Cambridge University Press, Cambridge.

NIELSEN, I., GUINET, Ph. & BARETTE-KUIPERS, T. 1983a. Studies in the Malesian, Australian and Pacific Ingeae (Leguminosae-Mimosoideae): the genera *Archidendropsis, Wallaceadendron, Paraserianthes, Pararchidendron* and *Serianthes* (1). *Bull. Mus. Nain. Hist. Nat., Paris, 4^e ser., 5, section B, Adansonia,* no 3, 303-329.

NIELSEN, I., GUINET, Ph. & BARETTE-KUIPERS, T. 1983b. Studies in the Malesian, Australian and Pacific Ingeae (Leguminosae - Mimosoideae): the genera *Archidendropsis, Wallaceodendron, Paraserianthes, Pararchidendron* and *Serianthes*. (2). *Bull. Mus. nain. Hist. nat. Paris, 4^e ser., 5, section B, Adansonia,* no 4, 335-360.

NOBLE, I.R. & SLATYER, R.O. 1980. The use of vital attributes to predict successional changes in plant communities subject to recurrent disturbance. *Vegetatio* 43, 5-21.

O' DOWD, D.J. & GILL, A.M. 1986. Seed dispersal syndromes in Australian *Acacia.* In: Seed dispersal, ed. D.R. Murray, pp. 87-121. Academic Press, Australia.

ONG, C. 1994. Alley cropping - ecological pie in the sky? *Agroforestry Today* 6, 8-10.

ONWUEME, I.C. & ADEGORAYE, S.A. 1975. Emergence of seedlings from different depths following high temperatures stress. *J. Agric. Sci.* 84, 525-528.

OSONUBI, O., MULONGOY, K., AWOTOYE, O.O., ATAYESE, M.O. & OKALI, D.U.U. 1991. Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal

fungi on drought tolerance of four leguminous woody seedlings. *Plant Soil* 136, 131-143.

PEMBERTON, R.W. & IRVING, D.W. 1990. Elaiosomes on weed seeds and the potential for myrmecochory in naturalized plants. *Weed Sci.* 38, 615-619.

PERRY, M.A., MICHELL, R.J., ZUTTER, B.R., GLOVER, G.R. & GJERSTAD, O.H. 1994. Seasonal variation in competitive effect on water stress and pine responses. *Can. J. For. Res.* 24, 1440-1449.

PETERSON, C.J. & FACELLI, J.M. 1992. Contrasting germination and seedling growth of *Betula alleghaniensis* and *Rhus typhina* subjected to various amounts and types of plant litter. *Amer. J. Bot.* 79, 1209-1216.

PIETERSE, P.J. 1986. Aspekte van die demografie van *Acacia longifolia* (Andr.) Willd in die Banhoekvallei in die Suidwes-Kaap. Unpublished M.Sc thesis, University of Stellenbosch, Stellenbosch.

PIETERSE, P.J. 1987. *Acacia longifolia* and its control: to burn or not to burn? *Veld & Flora* June 1987, 67-68.

PIETERSE, P.J. 1994. Foliar applied herbicides for chemical control of *Acacia longifolia* and *Paraserianthes lophantha*. *Applied Plant Science* 8, 54-56.

PIETERSE, P.J. & CAIRNS, A.L.P. 1986. The effect of fire on an *Acacia longifolia* seed bank in the south-western Cape. *S. Afr. J. Bot.* 52, 233-236.

PIETERSE, P.J. & CAIRNS, A.L.P. 1987. The effect of fire on an *Acacia longifolia* seed bank on the growth, mortality and reproduction of seedlings establishing after a fire in the South West Cape. *Appl. Plant Sci.* 1, 34-38.

PIETERSE, P.J. & MCDERMOTT, J.B. 1994. Season of application and glyphosate formulation as factors influencing the efficacy of glyphosate on phyllode bearing Australian acacias. *S. Afr. J. Plant Soil.* 11, 50-53.

POPAY, A.I. & ROBERTS, E.H. 1970. Factors involved in the dormancy and germination of *Capsella bursa-pastoris* (L.) Medik. and *Senecio vulgaris* L. *J. Ecol.* 58, 103-122.

PREECE, P.B. 1971. Contributions to the biology of mulga (*Acacia aneura*), II: germination. *Aust. J. Bot.* 19, 39-49.

QIU, J. & MOSJIDIS, J.A. 1993. Influence of seed weight and planting depth on common vetch establishment & growth. *Field Crops Res.* 33, 353-366.

QIU, J., MOSJIDIS, J.A. & WILLIAMS, J.C. 1994. Seedling growth as affected by seed weight in common vetch. *Agron. J.* 86, 251-255.

RADOSEVICH, S.R. & HOLT, J.S. 1984. Weed ecology: implications for vegetation management. Wiley, New York.

RICHARDSON, D.M. & COWLING, R.M. 1992. Why is Mountain Fynbos invasible and which species invade? In: Fire in South African Mountain Fynbos. Ecosystem, community and species response at Swartboschkloof, eds. B.W. van Wilgen, D.M. Richardson, F.J. Kruger & H.J. van Hensbergen. pp. 161-181. Ecological Studies 93, Springer Verlag, Berlin.

RICHARDSON, D.M. & VAN WILGEN, B.W. 1986. The effects of fire in felled *Hakea sericea* and natural fynbos and implications for weed control in mountain catchments. *S. Afr. Forestry J.* 139, 4-14.

RICHARDSON, D.M., MACDONALD, I.A.W., HOLMES, P.M. & COWLING, R.M. 1992. Plant and animal invasions. In: The ecology of fynbos. Nutrients, fire and diversity, ed. R.M. Cowling, pp. 271-308. Oxford University Press, Cape Town.

RIPPIN, M., HAGGAR, J.P., KASS, D.T. & KÖPKE, U. 1994. Alley cropping and mulching with *Erythrina poeppigiana* (Walp.) O.F. Cook and *Gliricidia sepium* (Jacq.) Walp.: effects on maize/weed competition. *Agroforestry Syst.* 25, 119-135.

ROKICH, D.P. & BELL, D.T. 1995. Light quality and intensity effects on the germination of species from the jarrah (*Eucalyptus marginata*) forest of Western Australia. *Aust. J. Bot.* 43, 169-179.

ROLSTON, M.P. 1978. Water impermeable seed dormancy. *Bot. Rev.* 44, 365-396.

ROMO, J.T. & EDDLEMAN, L.E. 1995. Use of degree-days in multiple temperature experiments. *J. Range Manage.* 48, 410-416.

SABIITI, E.N. & WEIN, R.W. 1987. Fire and *Acacia* seeds: a hypothesis of colonization success. *J. Ecol.* 74, 937-946.

SACKVILLE-HAMILTON, N.R. 1994. Replacement and additive designs for plant competition studies. *J. Appl. Ecol.* 31, 599-603.

SALISBURY, E.J. 1942. The reproductive capacity of plants; studies in quantitative biology. Bell, London.

SAS INSTITUTE INC. 1985. SAS users guide. Version 5. Carvy, NC. USA.

SASS, J.E. 1958. Botanical microtechnique. Iowa State Univ. Press, Iowa.

SCHÖNAU, A.P.G. & SCHULZE, R.E. 1984. Climatic and altitudinal criteria for commercial afforestation with special reference to Natal. *S. Afr. Forestry J.* 130, 10-18.

SEIWA, K. & KIKUZAWA, K. 1991. Phenology of tree seedlings in relation to seed size. *Can. J. Bot.* 69, 532-538.

SENARATNA, T. & McKERSIE, B.D. 1983. Dehydration injury in germinating soybean (*Glycine max* L. Merr.) seeds. *Plant Physiol.* 72, 620-624.

SEYDACK, A.H.W. & BEKKER, S.J. 1995. A review of fynbos catchment management policy. In: Managing fynbos catchments for water, eds. C. Boucher & C. Marais. Proceedings of a workshop held on 30 November 1993 at Stellenbosch, South Africa. *FRD Programme Report Series No.* 24, 9-34.

SHANMUGANATHAN, V. & BENJAMIN, L.R. 1992. The influence of sowing depth and seed size on seedling emergence time and relative growth rate in spring cabbage (*Brassica oleraceae* var. capitata L.). *Ann. Bot.* 69, 273-276.

SHARMA, M.L. 1973. Simulation of drought and its effect on germination of five pasture species. *Agron. J.* 65, 982-987.

SHAUGHNESSY, G.L. 1980. Historical ecology of alien woody plants in the vicinity of Cape Town, South Africa. Ph.D. thesis, University of Cape Town, Cape Town.

SHEA, S.R., McCORMICK, J. & PORTLOCK, C.C. 1979. The effect of fires on regeneration of leguminous species in the northern jarrah (*Eucalyptus marginata* Sm.) forests of Western Australia. *Aust. J. Ecol.* 4, 195-206.

SHERRY, S.P. 1971. The Black Wattle. (*Acacia mearnsii* De Wild.). University of Natal Press, Pietermaritzburg.

SIMON, E.W. 1984. Early events in germination. In: Seed physiology. Vol. 2. Germination and reserve mobilization, ed. D.R. Murray. pp. 77-115. Academic Press, Sydney.

SMITH, H. 1973. Light quality and germination: ecological implications. In: Seed ecology, ed. W. Heydecker. pp. 219-230. Butterworths, London.

SMITH, R.E. & BOSCH, J.M. 1989. A description of the Westfalia experiment to determine the influence of conversion of indigenous forest on water yield. *S. Afr. Forestry J.* 151, 26-31.

SMITH, R.E & SCOTT, D.F. 1992. The effects of afforestation on low flows in various regions of South Africa. *Water SA* 18, 185-194.

SNAYDON, R.W. 1991. Replacement or additive designs for competition studies? *J. Appl. Ecol.* 28, 930-946.

SNAYDON, R.W. 1994. Replacement and additive designs revisited: comments on the review paper by N.R. Sackville-Hamilton. *J. Appl. Ecol.* 31, 784-786.

SNAYDON, R.W. & SATORRE, E.H. 1989. Bivariate diagrams for plant competition data: Modifications and interpretation. *J. Appl. Ecol.* 26, 1043-1057.

SNEDECOR, G.W. & COCHRAN, W.G. 1980. Statistical methods. 7th ed. Iowa State University Press, Ames.

SPECHT, R.L. & CLIFFORD, H.T. 1991. Plant invasions and seed banks: control by water and nutrients. In: Biogeography of mediterranean invasions, eds. R.H. Groves & F. Di Castri, pp. 191-205. Cambridge University Press, Cambridge.

STIRTON, C. (Ed.). 1978. Plant invaders: beautiful but dangerous. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape Town.

STOCK, W.D. & ALLSOPP, N. 1992. Functional perspective of ecosystems. In: The ecology of Fynbos. Nutrients, fire and diversity, ed. R.M. Cowling. pp. 241-259, Oxford University Press, Cape Town.

STOCK, W.D., PATE, J.S. & DELFS, J. 1990. Influence of seed size and quality on seedling development under low nutrient conditions in five Australian and South African members of the Proteaceae. *J. Ecol.* 78, 1005-1020.

STUBBINGS, J.A. 1977. A.C.A.C.I.A. *S. Afr. Forestry J.* 102, 15-16.

STUBBINGS, J.A. & SCHÖNAU, A.P.G. 1983. Silviculture of black wattle. Forestry Handbook 81-90. SAIF.

TAYLOR, H.C. 1969. Pest-plants and nature conservation in the winter rainfall region. *J. Bot. Soc. S. Af.* 55, 32.

TAYLOR, H.C. 1978. Stinkbean. In: Plant invaders, beautiful but dangerous, ed. C.H. Stirton, pp. 64-67. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape Town.

TAYLORSON, R.B. 1982. Interaction of phytochrome and other factors in seed germination. In: The physiology and biochemistry of seed development, dormancy and germination, ed. A.A. Khan, pp. 323-346. Elsevier Biomedical Press, The Netherlands.

THOMPSON, K., GRIME, J.P. & MASON, G. 1977. Seed germination in response to diurnal fluctuations of temperature. *Nature* 267, 147-149.

TILANDER, Y. 1993. Effects of mulching with *Azadirachta indica* and *Albizzia lebbeck* leaves on the yield of sorghum under semi-arid conditions in Burkina Faso. *Agroforestry Syst.* 24, 277-293.

TOBIESSEN, P. & WERNER, M.B. 1980. Hardwood seedling survival under plantations of scotch pine and red pine in central New York. *Ecology* 61, 25-29.

TRABAUD, L. 1991. Is fire an agent favouring plant invasions? In: Biogeography of mediterranean invasions, eds. R.H. Groves & F. Di Castri, pp. 179-190. Cambridge University Press, Cambridge.

TRAN, V.N. 1979. Effects of microwave energy on the strophiole, seed coat and germination of *Acacia* seeds. *Aust. J. Plant Physiol.* 6, 277-287.

TRAN, V.N. & CAVANAGH, A.K. 1980. Taxonomic implications of fracture load and deformation histograms and the effects of treatments on the impermeable seed coat of *Acacia* species. *Aust. J. Bot.* 28, 39-51.

TRAN, V.N. & CAVANAGH, A.K. 1984. Structural aspects of dormancy. In: Seed physiology Vol. 2: Germination and reserve mobilization, ed. D.R. Murray, pp. 1-44. Academic Press, Australia.

TWAMLEY, B.E. 1967. Seed size and seedling vigor in birdsfoot trefoil. *Can. J. Plant Sci.* 47, 603-609.

VAN BAALEN, J. 1982. Population biology of plants in woodland clearings. Ph.D. thesis, Offsetdrukkerij, Alblasserdam.

VAN DEN BERG, M.A. 1973. Black and silver wattle intrusion. Official communication. Ref. 13/3/417/6 dated 13th August, 1973 from Director, Plant Protection Research Institute to Director, Wattle Research Institute.

VAN LILL, W.S., KRUGER, F.J. & VAN WYK, D.B. 1980. The effect of afforestation with *Eucalyptus grandis* Hill ex Maiden and *Pinus patula* Schlecht. Et Cham. on streamflow from experimental catchments at Mokobulaan, Transvaal. *J. Hydrol.* 48, 107-118.

VAN STADEN, J., MANNING, J.C. & KELLEY, K.M. 1989. Legume seeds - the structure:function equation. In: Advances in legume biology, eds. C.H. Stirton & J.L. Zaruchi. *Monogr. Syst. Bot. Missouri. Bot. Gard.* 29, 417-450.

VAN WILGEN, B.W & RICHARDSON, D.M. 1985. The effects of alien shrub invasions on vegetation structure and fire behaviour in South African fynbos shrublands: a simulation study. *J. Appl. Ecol.* 22, 955-966.

VAN WYK, D.B. 1987. Some effects of afforestation on streamflow in the Western Cape Province, South Africa. *Water SA*, 14, 31-36.

VER ELST, P. 1996. Indringerplanten in landbouwsystemen van de Westkaapprovinsie (Suid-Afrika). Een preliminaire studie. Eindverhandeling voorgedragen tot het behalen van de graad van Bio-ingenieur in de Landbouwkunde, Faculteit Landbouwkundige en Toegepaste Biologische Wetenskappen, Katholieke Universiteit Leuven, Leuven.

VERMEULEN, J.B., DREYER, M. & GROBLER, H. 1993. A guide to the use of herbicides. Fourteenth edition. Department of Agriculture, Republic of South Africa. Government Printer, Pretoria.

VERMEULEN, W.J. 1989. Guide on the control of Australian acacias. Pamphlet 365/8 - Plant invaders. Department of Environment Affairs.

VERSFELD, D.B. & VAN WILGEN, B.W. 1986. Impact of woody aliens on ecosystem properties. In: The ecology and management of biological invasions in Southern Africa, eds. I.A.W. Macdonald, F.J. Kruger & A.A. Ferrar. Proceedings of the National Synthesis Symposium on the Ecology of Biological Invasions. pp. 295-303. Oxford University Press, Cape Town.

VINCENT, E.M. & CAVERS, P.B. 1978. The effect of wetting and drying on the subsequent germination of *Rumex crispus*. *Can. J. Bot.* 56, 2207-2217.

WALLER, D.M. 1988. Plant morphology and reproduction. In: Plant reproductive ecology: patterns and strategies, eds. J. Lovett Doust & L. Lovett Doust. pp. 203-227. Oxford University Press, New York.

WELLER, S.G. 1985. Establishment of *Lithospermum caroliniensis* on sand dunes: the role of nutlet mass. *Ecology* 66, 1893-1901.

WERKER, E. 1980/81. Seed dormancy as explained by the anatomy of embryo envelopes. *Isr. J. Bot.* 29, 22-44.

WITHERS, J.R. 1979. Studies on the status of unburnt *Eucalyptus* woodland at Ocean Grove, Victoria. V. The interactive effects of droughting and shading on seedlings under competition. *Aust. J. Bot.* 27, 285-300.

WILLIAMS, R.D. 1980. Moisture stress and hydration-dehydration effects on hemp sesbania (*Sesbania exaltata*) seed germination. *Weed Sci.* 21, 322-324.

WULFF, R.D. 1986. Seed size variation in *Desmodium paniculatum*. II. Effects on seedling growth and physiological performance. *J. Ecol.* 74, 99-114.

The method of analysis of an experiment involving mixtures

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Introduction

This document describes the analysis of an experiment conducted by P.J. Pieterse, (at the time) of the Plant Protection Unit, Stellenbosch. The purpose of the document is to supply the background detail required for writing the experiment up for publication.

The basic purpose of the experiment was to obtain comparative information on the relative aggressiveness of three encroaching pest plant species, namely *Paraserianthus lophanta*, *Acacia melanoxylon*, and *Acacia mearnsii*. For convenience these names are abbreviated to P, X, and R in what follows.

The treatment design was a 6×7 factorial; 48 seeds were planted in a pot at one of six depths (10, 30, 30, 90, 120, 150 mm) in one of the seven possible mixtures amongst the species (P, X, R, PX, PR, XR, PXR). In the case of the uniblends (P, X, R) 48 seeds of a single species were planted. In the case of the biblends (PX, PR, XR) 24 seeds of each of two species were mixed and sown in a pot, while in the case of the triblend (PXR) 16 seeds from each species made up the mixture.

Two replicates of the 6×7 treatment combinations were arranged as a randomised block design in the glass house.

Two response variables were measured on each pot; the number of seedlings which established themselves (of 48 possible) and the dry mass of the plants in a pot two months after planting. The latter variable may be analysed by fitting conventional linear models, but the former response is binary and discrete, meaning that some special technique must be invoked to obtain valid analyses corresponding to those obtained for dry mass. We choose to fit a generalised linear model, Nelder and Wedderburn (1972) and McCullagh and Nelder (1983). It is convenient to display the analysis of dry plant material prior to the analysis of germination percentage, to motivate the model fitted in the second case. For both variables the response in each pot was recorded, but this was also broken down according to species.

Problems with the interpretation of the mixture-by-depth interaction observed, as well as with the modelling of the decrease in variation observed with increasing depth of sowing motivated us to present separate analyses for each depth.

The analysis of dry matter

THE ANALYSIS

The raw data is listed in Table 1. The model fitted is explained in Tables 2 and 3. The model for the plot totals is explained in Table 2 and that of the species responses in Table 3.

The analysis of the total response in each pot is given in Table 5. This analysis is optimum when plot totals are uncorrelated with common variance, write σ^2 for this quantity. Since the model in Table 2 has no common mean there are seven degrees of freedom (df's) for mixtures. The first three of these describe the uniblends. A further three degrees of freedom are associated with deviation of the responses of the biblends from the

response predicted from the uniblend responses and the last is associated with the deviation of the triblend response from a response predicted from the biblend responses.

Table 1 The dry matter produced by 48 seeds in two months

Mixture		Depth(mm)											
		10		30		60		90		120		150	
P		10.63	11.63	6.44	7.40	7.25	10.13	8.18	7.96	5.55	6.29	0.00	1.30
X		1.82	1.70	2.06	1.14	0.21	0.15	0.00	0.00	0.00	0.00	0.00	0.00
R		6.20	3.34	1.37	1.91	0.82	0.89	0.62	0.48	0.13	0.09	0.00	0.13
PX:	P	5.54	3.92	6.29	6.86	5.73	4.25	4.74	4.54	1.46	4.42	0.30	0.00
	X	0.96	0.88	0.61	0.56	0.30	0.25	0.00	0.00	0.00	0.00	0.00	0.00
PR:	P	4.08	4.31	4.95	4.26	6.12	6.25	2.69	3.73	2.71	2.34	0.00	0.00
	R	0.05	0.53	1.20	0.99	1.62	1.04	0.00	0.50	0.04	0.03	0.00	0.00
XR:	X	0.92	0.64	0.65	0.84	0.48	0.46	0.14	0.00	0.00	0.00	0.00	0.00
	R	1.19	0.99	1.02	0.65	0.39	1.83	0.41	0.52	0.00	0.11	0.00	0.00
PXR:	P	3.07	3.83	2.80	3.64	3.48	2.91	3.95	2.94	1.63	2.03	0.00	0.00
	X	0.45	0.67	0.30	0.57	0.25	0.30	0.03	0.11	0.00	0.00	0.00	0.00
	R	0.69	0.89	0.44	0.69	0.42	0.80	0.36	0.14	0.15	0.00	0.00	0.00

Notes: 1) P = *Paraserianthus lophanta*
X = *Acacia melanoxylon*
R = *Acacia mearnsii*

2) The response in the first block is on the left in column, the response in the second block is on the right.

Table 2: A model for the total of the observations on a plot.

Mixture	Model
P	μ_P
X	μ_X
R	μ_R
PX	$\mu_{PX} = \frac{1}{2}(\mu_P + \mu_X) + \frac{1}{2}\delta_{PX}$
PR	$\mu_{PR} = \frac{1}{2}(\mu_P + \mu_R) + \frac{1}{2}\delta_{PR}$
XR	$\mu_{XR} = \frac{1}{2}(\mu_X + \mu_R) + \frac{1}{2}\delta_{XR}$
PXR	$\mu_{PXR} = \frac{1}{3}(\mu_P + \mu_X + \mu_R) + \frac{1}{6}(\delta_{PX} + \delta_{PR} + \delta_{XR}) + \frac{1}{3}\delta_{PXR}$

The model equation for a biblend is motivated by calculating the difference between twice the response of a biblend and the sum of the responses of uniblends on a full-size plot, i.e. the result (e.g. δ_{PX}) is expressed in full-plot terms. The model equation for the triblend is derived by comparing three times the triblend response to the total of the biblend responses, i.e. δ_{PXR} is also expressed in full-plot terms. A non-zero value for this parameter indicates that the triblend is not acting as though three biblend systems were pooled in equal

proportions. These parameter estimates, with standard errors, are given in Table 6 in the case of planting at a depth of 30 mm.

Table 3 A model for the observations per species in the blends

Mixture		Model
P		μ_P
X		μ_X
R		μ_R
PX:	P	$\mu_{PX:P} = \frac{1}{2}\mu_P + \frac{1}{2}\delta_{PX:P}$
	X	$\mu_{PX:X} = \frac{1}{2}\mu_X + \frac{1}{2}\delta_{PX:X}$
PR:	P	$\mu_{PR:P} = \frac{1}{2}\mu_P + \frac{1}{2}\delta_{PR:P}$
	R	$\mu_{PR:R} = \frac{1}{2}\mu_R + \frac{1}{2}\delta_{PR:R}$
XR:	X	$\mu_{XR:X} = \frac{1}{2}\mu_X + \frac{1}{2}\delta_{XR:X}$
	R	$\mu_{XR:R} = \frac{1}{2}\mu_R + \frac{1}{2}\delta_{XR:R}$
PXR:	P	$\mu_{PXR:P} = \frac{1}{3}\mu_P + \frac{1}{6}\left(\delta_{PX:P} + \delta_{PR:P}\right) + \frac{1}{3}\delta_{PXR:P}$
	X	$\mu_{PXR:X} = \frac{1}{3}\mu_X + \frac{1}{6}\left(\delta_{PX:X} + \delta_{XR:X}\right) + \frac{1}{3}\delta_{PXR:X}$
	R	$\mu_{PXR:R} = \frac{1}{3}\mu_R + \frac{1}{6}\left(\delta_{PR:R} + \delta_{XR:R}\right) + \frac{1}{3}\delta_{PXR:R}$

The model in Table 3 is derived from that in Table 2. That is, if one adds up the model equations for the response of a species in a blend in Table 3, one gets the model for the plot total in Table 2. The following additional definitions hold.

Table 4: The relationships between some of the symbols in Tables 2 and 3

$$\delta_{PX} = \delta_{PX:P} + \delta_{PX:X}$$
$$\delta_{PX} = \delta_{PX:P} + \delta_{PX:X}$$
$$\delta_{PX} = \delta_{PX:P} + \delta_{PX:X}$$
$$\delta_{PXR} = \delta_{PXR:P} + \delta_{PXR:X} + \delta_{PXR:R}$$

The ANOVA corresponding to the model in Table 3 is given in Table 7 and parameter estimates in Table 8.

The analysis of the component responses is complicated by the fact that one has responses based on "plots" of different sizes. As stated above, the model for the total (pot) yields assumes uncorrelated observations with common variance, σ^2. In the case of a biblend, with component responses are x and x', the model states that $\mathrm{Var}(x + x') = \sigma^2$. Expanding the left-hand side of this equation and assuming uncorrelated components (probably an unreeasonable assumption) with common variance, it turns out that the common variance is $\sigma^2/2$. For a triblend, under the same assumptions, the common variance of a component

must be $\sigma^2/3$ if the model for the components is to be consistent with the model for the pot totals. Thus, a weighted analysis of variance is called for.

RESULTS

An initial two-factor analysis, blends by depth, suggested interaction between these two factors and closer examination of the data suggested the possibility of a decreasing error variance with increasing depth. Both of these considerations lead one to consider a separate analysis for each depth.

Beginning with an analysis of the full-plot yields, the sums of squares displayed in Table 5 suggest that the response of a triblend may be predicted by simply averaging the responses of the three uniblends. Examining the biblend effects next, an exhaustive analysis (fitting parameters in various sequences) suggested that there is a non-zero biblend effect (interaction) between the species P and R for the first three depths of planting and between P and X at the second depth, 30 mm. The PR effect is not significant at this depth when fitted in the order shown in Table 5, but if this effect is fitted after P, X, PX and R then the significance level becomes 0.0491, suggesting that this term is needed at this depth. The biblend effect between X and R was not significant at any depth regardless of order of fitting tried.

The experiment described here was a little too small for statistical purposes. This is to be seen in the fact that the terms P, R and particularly X were sometimes (always) not significantly different from zero even though the species concerned did grow.

The information obtained in the experiment suggests very vigorous growth for the species P and an interaction between this species and the species R at shallow depths. At an intermediate depth, P also interacts with X.

Table 5: Significance levels (mean squares in the case of the error line) of a sequential analysis of variance of dry material yield for each of six depths (in mm). Parameters were fitted in the sequence shown. All significance levels less than 0.00005 are rounded up to 0.0001.

Source of variation	df	10	30	60	90	120	150
Blocks	1	0.5066	0.5791	0.5760	0.9274	0.2684	0.4418
P	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0699
R	1	0.0008	0.0009	0.0130	0.1531	0.9637	0.7715
PR	1	0.0116	0.2703	0.0336	0.1245	0.5268	0.3124
X	1	0.0971	0.0006	0.2995	0.2559	0.9965	0.7438
PX	1	0.6036	0.0019	0.5440	0.1526	0.9905	0.5740
XR	1	0.2107	0.7343	0.3274	0.3482	0.9848	0.8889
PXR	1	0.4225	0.2920	0.4205	0.1018	0.9374	0.8729
Error	6	1.0598	0.3958	1.0258	0.3164	0.6364	0.1345

Table 6 shows that seeds planted at 30 mm have a dry material yield of 1.6 ± 0.445 units in the case of R and X but 6.9 ± 0.445 (more than 4 times as much) in the case of P. When planted together on two pots, the joint yield of P and R is 2.8 ± 1.09 more than the yield one would get if these two species were planted each on its own pot. In the case of P and X the increase is 5.8 ± 1.09.

Table 6: Full-plot parameter estimates at a depth of 30 mm

Parameter	Estimate	Standard error	Significance Level
P	6.9	0.445	0.0001
R	1.6	0.445	0.0103
PR	2.8	1.09	0.0403
X	1.6	0.445	0.0114
PX	5.8	1.09	0.0018
XR	-0.8	1.09	0.9439
PXR	-1.8	1.54	0.2920

At 10 mm the PR estimate is -6.5 ± 1.78 and at 60 mm it is 5.5 ± 1.75. Apparently, there is a disadvantage when planting these two species together at a shallow depth, but this disadvantage decreases and becomes an advantage as the depth of planting is increased.

The analysis above does not reveal the reaction of one species when planted with one or more other species. The component analysis must be used to reveal this information.

Table 7: Significance levels (mean squares in the case of the error line) of a sequential analysis of variance of dry material yield, by species, for each of six depths (in mm). Parameters were fitted in the sequence shown. All significance levels less than 0.00005 are rounded up to 0.0001.

Source of variation	df	10	30	60	90	120	150
Blocks	1	0.4085	0.5067	0.5276	0.9209	0.2924	0.3011
P	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0222
R	1	0.0001	0.0001	0.0022	0.0220	0.7776	0.8328
PR:P	1	0.0573	0.6141	0.0136	0.0259	0.4076	0.0909
PR:R	1	0.0114	0.4278	0.2341	0.8087	0.9879	0.8331
X	1	0.0011	0.0001	0.2515	0.8100	1.0000	1.0000
PX:P	1	0.2122	0.0001	0.4246	0.0128	0.9745	0.2601
PX:X	1	0.8934	0.5183	0.8672	0.9833	1.0000	1.0000
XR:X	1	0.8400	0.8570	0.4838	0.7820	1.0000	1.0000
XR:R	1	0.0341	0.9644	0.2678	0.5416	0.9745	0.8458
PXR:P	1	0.2586	0.0708	0.2462	0.0066	0.9843	0.7104
PXR:X	1	0.9870	0.9743	0.9520	0.8542	1.0000	1.0000
PXR:R	1	0.5356	0.7629	0.6343	0.9633	0.9155	1.0000
Error	11	0.7155	0.2887	0.8425	0.2770	0.7739	0.0775

The lines drawn across the columns in Table 7 are drawn at the same place as in Table 5. Note the change in error estimate between the two analyses. Significant effects below this line are indicated by shading. Some of these differences between the two tables are due to the change in the error estimate. For example, the parameter estimate for X is the same in the two analyses but in the case of the responses when planting at 10 mm the estimate (1.76) is divided by 1.0598 (based on 6 df) in Table 5 and 0.7155 (based on 11 df) in Table 7, with a consequent change in significance level from 0.0971 to 0.0011. Other differences between the two tables may be due to a small effect being added to a large effect so that, in the total, the small effect masks the large effect. Such a case may be concerned

for XR at 10 mm, which has a significance level of 0.2107 in Table 5. In Table 7 the significance levels for XR:X and XR:R are 0.8400 and 0.0341 respectively; a total of -2.79 is subdivided into -0.2 and -2.59 in the full analysis.

Table 8: Parameter estimates (unrounded) fitted to the component measurements of dry material made when seeds were planted at various depths (in mm) in various blends. Shaded estimates have a significance level (in a test for zero) less than 5%.

Parameter	10	30	60	90	120	150
P	11.13	6.92	8.69	8.07	5.92	0.65
R	4.77	1.64	0.855	0.55	0.11	0.065
PR:P	-2.74	2.29	3.68	-1.65	-0.87	-0.65
PR:R	-3.74	0.55	1.805	-0.05	-0.04	-0.065
X	1.76	1.60	0.18	0.00	0.00	0.00
PX:P	-1.67	6.23	1.29	1.21	-0.04	-0.35
PX:X	0.08	-0.43	0.37	0.00	0.00	0.00
XR:X	-0.20	-0.11	0.76	0.14	0.00	0.00
XR:R	-2.59	0.03	1.415	0.38	0.00	0.065
PXR:P	1.425	-1.52	-1.59	2.485	0.025	0.15
PXR:X	-0.02	-0.025	0.08	0.14	0.00	0.00
PXR:R	0.765	-0.235	-0.635	0.035	0.135	0.00

The interpretation of Table 8 is little different from the interpretation given for the analysis of pot totals. It seems that P produces (relatively) large quantities of dry matter. The species R produces the second largest amount and X the third, both at shallow depths. There is a biblend effect when P and R are planted together, in that the blend under-produces at shallow depths and over-produces at intermediate depths. In regard to PX, there is a curiosity at a depth of 30 mm. In uniblend, P produced 6.44 and 7.44 units in the two blocks of the experiment concerned (Table 1). In biblend (half the number of seeds) with X, P produced 6.29 and 6.86 units – almost as much as in the case of the uniblend. The comparable numbers for X are 2.06 and 1.14 and 0.61 and 0.56, indicating a slight (and non-significant) under-production in biblend.

An important point to note in the analysis is the remarkable consistency of the performance of X in blends with its performance alone. If this observation is not fortuitous then it means that in its performance this species is insensitive to the presence or absence of the other two species.

The stochastic properties of all measurements was taken to be that these are uncorrelated with common variance, σ^2 for a measurement on a uniblend, $\sigma^2/2$ for a measurement on a biblend and $\sigma^2/3$ for a measurement on a triblend. These assumptions may be checked by partitioning the degrees of freedom for error according to blend type. Thus there are 6 df for uniblends (Table 5) 3 for biblends and 2 for triblends. Table 9 lists the residual error (block by treatment interaction) partitioned according to the plot size of a response. These estimates are pooled in the last line (e.g. $[6\times1.0598 + 3\times0.4030 + 2\times0.1514]\div11 = 0.7155$) to correspond with the error estimates given in Table 7. The full-plot error *could* be further partitioned into three components, according to the number of species in a blend, but there are too few degrees of freedom for the available information to be useful, so we resist the temptation to make the split of 6 degrees of freedom into 2, 2 and 1.

Table 9 Estimating σ^2 from three sources

Source	Depth (mm)					
	10	30	60	90	120	150
Uniblends	1.0598	0.3958	1.0258	0.3164	0.6364	0.1345
Biblends	0.4030	0.1547	0.8048	0.0657	1.4839	0.0150
Triblends	0.1514	0.1684	0.3490	0.4756	0.1212	0.0000
Pooled	0.7155	0.2887	0.8425	0.2770	0.7739	0.0775

Table 9 suggests that there is a decrease in error variance with increase in number of species present in a mixture. A possible explanation for this is that the observations in pots are negatively correlated. In other words, if one species shows overwhelming growth, the other species will probably not have access to the resources needed for their growth, hence their growth will be stunted. On the other hand if, for some reason, growth of the dominant species is stunted in a pot, the other species will have the opportunity to grow. A superior model might therefore allow for a correlation between observations on the same pot. The current experiment is too small for the fitting of such a model so we do more here than note that better may be possible. In addition to the size of the experiment, although the software exists for the fitting of such models, we do not have access to this software.

Our final conclusions are that for planting depths of 30 mm and more the difference between *Paraserianthus lophanta* and the *Acacia* species is overwhelming. Secondly, the pattern of blending is different at 10 mm to that at the greater depths. This may be important if seeds are not "planted" at depths under natural conditions. Our analysis was guided to some extent by a pre-print copy of Chapter 6 of Federer (1993) and by Federer and Raghavarao (1987).

The analysis of seedling establishment proportions

The data is listed in Table 10.

Table 10 The number of seedlings established in a mixture of 48 seeds planted.

Mixture	Depth (in mm)											
	10		30		60		90		120		150	
P	30	35	21	29	32	24	29	30	21	28	0	5
X	43	42	20	34	2	2	0	0	0	0	0	0
R	43	45	42	44	30	30	11	6	1	4	0	1
PX:P	18	20	17	19	12	10	10	11	4	14	1	0
PX:X	24	22	19	22	12	7	0	0	0	0	0	0
PR:P	11	16	14	15	14	14	14	13	8	11	0	1
PR:R	24	21	23	22	22	18	16	15	1	0	0	0
XR:X	19	20	24	19	10	10	0	2	0	0	0	0
XR:R	22	24	22	24	16	23	7	12	0	3	0	0
PXR:P	10	7	11	11	9	11	9	9	4	4	0	0
PXR:X	15	16	15	14	9	9	1	1	0	0	0	0
PXR:R	13	16	16	16	14	13	6	6	4	0	0	0

The **X** matrix used in the analysis of a continuous variable may be used to analyse data on a discrete variable using a generalised linear model. The only modification necessary is that the uniblend effects are not in themselves estimable, although contrasts amongst these effects are estimable. The contrasts used are P vs R and X vs P and R. The matrix used for the analysis of full-plot treatments responses is shown below. After the first column (the block contrast) the columns are in the order PvsR, PR (the biblend effect), XvsP&R, PX, XR and PXR (the triblend effect).

$$\mathbf{X} = \begin{bmatrix} 6 & 6 & 0 & -6 & 0 & 0 & 0 \\ 6 & 0 & 0 & 12 & 0 & 0 & 0 \\ 6 & -6 & 0 & -6 & 0 & 0 & 0 \\ 6 & 3 & 0 & 3 & 3 & 0 & 0 \\ 6 & 0 & 3 & -6 & 0 & 0 & 0 \\ 6 & -3 & 0 & 3 & 0 & 3 & 0 \\ 6 & 0 & 1 & 0 & 1 & 1 & 2 \\ -6 & 6 & 0 & -6 & 0 & 0 & 0 \\ -6 & 0 & 0 & 12 & 0 & 0 & 0 \\ -6 & -6 & 0 & -6 & 0 & 0 & 0 \\ -6 & 3 & 0 & 3 & 3 & 0 & 0 \\ -6 & 0 & 3 & -6 & 0 & 0 & 0 \\ -6 & -3 & 0 & 3 & 0 & 3 & 0 \\ -6 & 0 & 1 & 0 & 1 & 1 & 2 \end{bmatrix}.$$

The program PC-PLUM was used in the analysis. (The original program, PLUM, was written by Peter McCullagh, currently Professor of Statistics at the University of Chicago. It was adapted to run on IBM PC compatibles under MS-DOS by myself.) The analysis of the full-plot responses is shown in Table 11.

Table 11 The significance levels of terms of a model fitted sequentially to plot-total data on the number of seedlings establishing themselves in various mixtures of three species planted at one of six depths. Non-convergent models are marked "nc".

	df	10	30	60	90	120	150
Blocks	1	0.2203	0.0230	0.3954	0.8675	0.0264	0.0237
PvR	1	0.0001	0.0001	0.0924	0.0001	0.0001	0.0057
PR	1	0.0076	0.6187	0.0001	0.0001	0.0011	0.8959
XvP&R	1	0.0916	0.0118	0.0001	0.0001	0.0001	0.0283
PX	1	0.9381	0.0001	0.0340	0.9537	0.0437	nc
XR	1	0.4691	0.0003	0.0001	nc	nc	nc
PXR	1	0.3376	0.6906	0.1183	nc	nc	nc
Residual	6	0.9598	0.1827	0.3220	0.0008*	0.0901*	0.5376*

* The residual deviance of the last convergent model. The residual degrees of freedom change accordingly. These are 6, 6, 6, 8, 8 and 9 respectively.

Table 11 suggests that in the cases were model-convergence allows its estimation, there is no need to take the triblend effect into account. The evidence in regard to biblend effects is confusing. At 30 and 60 mm a triblend effect between X and R seems necessary, but not at 10 mm. This effect seems relevant at the depths of 30, 60 and 120 in the case of PX, but

not in the case of 10 and 90 mm. The effect PR appears to be needed at 10, 60, 90 and120 mm, but not at 30 and 150 mm.

It is difficult to interpret the contrasts amongst the uniblend effects.

The last line in the table, the line labelled "Residual" is a test of the goodness of fit of the model used. In all cases except the depth of 90 mm the model seems acceptable.

The X matrix for the analysis of responses by species is shown below.

$$\mathbf{X} = \begin{bmatrix}
6 & 6 & 0 & 0 & -6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
6 & 0 & 0 & 0 & 12 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
6 & -6 & 0 & 0 & -6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
3 & 3 & 0 & 0 & -3 & 3 & 0 & 0 & 0 & 0 & 0 & 0 \\
3 & 0 & 0 & 0 & 6 & 0 & 3 & 0 & 0 & 0 & 0 & 0 \\
3 & 3 & 3 & 0 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
3 & -3 & 0 & 3 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
3 & 0 & 0 & 0 & 6 & 0 & 0 & 3 & 0 & 0 & 0 & 0 \\
3 & -3 & 0 & 0 & -3 & 0 & 0 & 0 & 3 & 0 & 0 & 0 \\
2 & 2 & 1 & 0 & -2 & 1 & 0 & 0 & 0 & 2 & 0 & 0 \\
2 & 0 & 0 & 0 & 4 & 0 & 1 & 1 & 0 & 0 & 2 & 0 \\
2 & -2 & 0 & 1 & -2 & 0 & 0 & 0 & 1 & 0 & 0 & 2 \\
-6 & 6 & 0 & 0 & -6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-6 & 0 & 0 & 0 & 12 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-6 & -6 & 0 & 0 & -6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-3 & 3 & 0 & 0 & -3 & 3 & 0 & 0 & 0 & 0 & 0 & 0 \\
-3 & 0 & 0 & 0 & 6 & 0 & 3 & 0 & 0 & 0 & 0 & 0 \\
-3 & 3 & 3 & 0 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-3 & -3 & 0 & 3 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-3 & 0 & 0 & 0 & 6 & 0 & 0 & 3 & 0 & 0 & 0 & 0 \\
-3 & -3 & 0 & 0 & -3 & 0 & 0 & 0 & 3 & 0 & 0 & 0 \\
-2 & 2 & 1 & 0 & -2 & 1 & 0 & 0 & 0 & 2 & 0 & 0 \\
-2 & 0 & 0 & 0 & 4 & 0 & 1 & 1 & 0 & 0 & 2 & 0 \\
-2 & -2 & 0 & 1 & -2 & 0 & 0 & 0 & 1 & 0 & 0 & 2
\end{bmatrix}$$

The analysis using this matrix is given in Table 12. In this analysis it appears as though the triblend effect is sometimes required in an appropriate model, particularly the P component of this effect. In the XR biblend effect the R component is almost always needed, the exception being at 10 mm, while for the PX biblend effect the P componenbt is almost never needed, compared to the R component which is always indicated. For the PR effect, P is not needed in the case of depths of 30 and 150 mm while the R component is not needed at depths of 10 and 120 mm.

Table 12 The significance levels of terms of a model fitted sequentially to data per species on the number of seedlings establishing themselves in various mixtures of three species planted at one of six depths. Non-convergent models are marked "nc".

	df	10	30	60	90	120	150
Blocks	1	0.2244	0.0025	0.3146	0.9105	0.0214	0.0018
PvR	1	0.0001	0.0001	0.0078	0.0001	0.0001	0.0063
PR:P	1	0.0001	0.3132	0.0595	0.0018	0.0097	0.6140
PR:R	1	0.6360	0.0259	0.0001	0.0001	0.5547	nc
XvP&R	1	0.1452	0.0019	0.0001	0.0001	0.0001	nc
PX:P	1	0.5169	0.4829	0.2322	0.1530	0.0042	nc
PX:R	1	0.0128	0.0115	0.0368	0.0406	nc	nc
XR:X	1	0.4016	0.0002	0.0016	0.5701	nc	nc
XR:R	1	0.1042	0.0024	0.0001	0.0007	nc	nc
PXR:P	1	0.0127	0.9716	0.0832	0.0131	nc	nc
PXR:X	1	0.1357	0.2658	0.0541	nc	nc	nc
PXR:R	1	0.7099	nc	0.0606	nc	nc	nc
Residual	11	0.0441	0.0474*	0.2004	0.7392*	0.0746*	0.9493*

* The residual deviance of the last convergent model. The residual degrees of freedom change accordingly. These are 11, 12, 11, 13, 17 and 20 respectively.

Overall the interpretation of the analysis of this data set is not easy in the absence of knowledge of the characteristics of the species concerned.

Reference

Federer, W.T. and Raghavarao, D., 1987: Response models and minimal designs for mixtures of *n* of *m* items useful for intercropping and other investigations. *Biometrika* 74, 571-577.

McCullagh, P. and Nelder, J.A., 1983: *Generalized linear models*. Chapman and Hall, London.

Nelder, J.A. and Wedderburn, R.W.M., 1972: Generalized linear models. *J. Roy. Statist. Soc.* A 135, 370-384.

Randall, J.H., 1989: The analysis of sensory data by generalized linear model. *Biom. J.* 31, No. 7, 781-793